MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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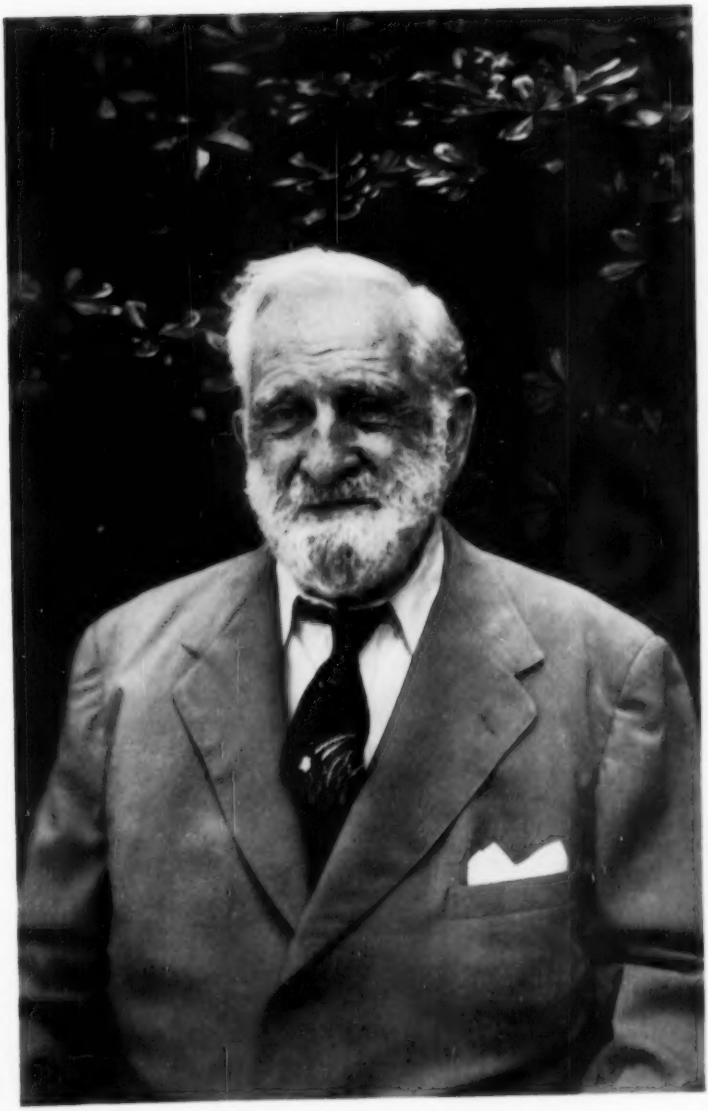

Dr. W. A. Murrill

DR. W. A. MURRILL

The establishment of MYCOLOGIA by the New York Botanical Garden in 1909 was due in very large degree to the energy and initiative of Dr. W. A. Murrill, who edited the journal until 1924. In recent years, Dr. Murrill has resided in Gainesville, where he has been associated with the University of Florida. Now in his 87th year, he still maintains an active interest in the fungi, exhibited in his writings and his extensive correspondence from all parts of the world.

On the occasion of his 86th birthday, October 13, 1955, the Department of Botany at Gainesville honored him with a birthday party attended by many of his friends as well as by the President of the University, the Provost of Agriculture and the Directors of the Experiment Stations.

His health has been excellent. He continues to observe and collect the fleshy fungi of the area and, unlike many of his youthful contemporaries, reads without glasses. As an avocation, he is an ardent observer of bowling.

The photograph here reproduced was taken by Dr. George F. Weber in July 1955. It is believed that Dr. Murrill's many friends will be glad to see it reproduced in the journal so largely associated with him.

MYCOLOGIA

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VOL. XLVIII JULY–AUGUST, 1956 NO. 4

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON CULTURAL CHARACTERS OF FUSARIUM SPECIES

ANNA T. ZACHARIAH,[1] H. N. HANSEN, AND WILLIAM C. SNYDER

(WITH 1 FIGURE)

Cultural variation in *Fusarium* species and the resultant complications in the problem of their taxonomy have been recognized by several investigators (Harter (**3**, **4**), Padwick (**10**), Snyder and Hansen (**13**), Miller (**6**, **7**, **8**), Oswald (**9**), and Subramanian (**16**)). The junior authors (**11**, **12**, **14**), in advocating a species concept modifying the Wollenweber and Reinking system (**18**), revised the genus, reducing the species to nine. They have also shown that such characters as color, zonation, type of colony, presence or absence of sporodochia, size, shape and septation of macroconidia and even the occurrence of a perithecial stage cannot be employed successfully in taxonomy unless these fungi are grown in adequate light. The advantages of culturing fungi on natural media under natural environmental conditions have also been discussed (Snyder and Hansen, **15**). The investigation reported here was taken up to determine the conditions that would help to maintain *Fusarium* cultures as near the natural clone as possible.

EXPERIMENTAL PROCEDURE

One clone each of eight of the nine species of *Fusarium* (new species concept) was used in this study. *Fusarium nivale* was not included. Single spore cultures of all species were made on the same day, following

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the method used by Hansen and Smith (1). Two percent potato-dextrose agar was selected as the medium on the basis of a preliminary experiment in which potato-dextrose agar, Czapek's agar and water agar slants with sterilized pea straw spread over the surface were compared. (These cultures had been exposed to a regular fluctuation in temperature, humidity and light, the temperatures fluctuating between 15° and 25° C, or 25° and 35° C, or remaining constant at 25° C.) The tubes, after inoculation, were capped with cigarette paper to prevent culture mite contamination (Hansen and Snyder, 2). The cultures were arranged in a single layer in climate chambers adjusted to the following conditions:

(1) fluctuating light and fluctuating temperature (darkness 10 hours, temperature 15° C; light 14 hours, temperature 25° C);
(2) fluctuating light and fluctuating temperature (darkness 12 hours, temperature 25° C; light 12 hours, temperature 30° C);
(3) continuous light and temperature fluctuating every 12 hours, 15° C and 25° C;
(4) fluctuating light (12 hours dark and 12 hours light) and continuous temperature 20° C;
(5) continuous light and continuous temperature 20° C;
(6) continuous darkness and continuous temperature 20° C.

The change of temperature in series 1, 2 and 3 was gradual. A set of cultures was also exposed to diurnal conditions of light and temperature in the room and another set outdoors on the ledge of a north window.

RESULTS

Marked differences in cultural characters were observed in these cultures as indicated in TABLE I and FIG. 1.

Observations made on the outdoor cultures are not indicated in the table as these cultures were badly inhibited in growth, the January temperatures in Berkeley having fluctuated a great deal, even dropping to freezing point on some nights.

All the species studied showed a more abundant mycelial growth in the dark than in the light, although this difference was not so apparent in *Fusarium tricinctum* as in other species.

Well-marked zonation was observed in all species during the earlier stages of growth in cultures exposed to fluctuating light and/or fluctuating temperature but this zonation effect was masked in some cultures by later aerial mycelial growth. However, pronounced zonation persists

and is characteristic of *Fusarium solani, Fusarium rigidiuscula* and *Fusarium roseum* in cultures grown in fluctuating light or fluctuating temperature (FIG. 1). *Fusarium oxysporum* also showed pronounced zonation in series 4, i.e., fluctuating light and constant temperature.

TABLE I

COMPARATIVE DEVELOPMENT OF CULTURES OF 8 FUSARIUM SPECIES AFTER 30 DAYS' GROWTH UNDER DIFFERENT CONDITIONS OF LIGHT AND TEMPERATURE (see p. 460)

Fungus species and key to Fig. 1		1	2	3*	4*	5*	6*	Room
F. moniliforme M	A.M.	++	scant	++	++	scant	++	++
	Z	++	++					
	Sp	+	+	+	+	+		+
	P	+	++	++		++		
F. solani S	A.M.	scant	+	scant	+	scant	+	+
	Z	++	++	++	++			+
	Sp	++	++	++	++	++	++	++
	P							
F. oxysporum O	A.M.	scant	scant	scant	+	scant	+	+
	Z	+		+	++			
	Sp	+	+	+	+	++		+
	P	++	++	++		++		
F. tricinctum T	A.M.	+	+	+	+	+	+	+
	Z	+						
	Sp							
	P							
F. rigidiuscula Ri	A.M.	scant	scant	scant	scant	scant	+	+
	Z	+	+	+	+			+
	Sp	++	++	++	++	++		++
	P							
F. roseum Ro	A.M.	++	++	++	++	++	++	++
	Z	++	+	++	+			
	Sp							
	Perithecia			+	++	+	primordia	+
F. episphaeria E	A.M.	+	+	+	+	+	++	+
	Z	+						
	Sp	++	++	++	+	+		+
	P							
F. lateritium L	A.M.	++	++	++	++	++	++	++
	Z							
	Sp							
	P							

* Shown in Fig. 1.
A.M. = Aerial mycelium.
Z = Zonation.
Sp = Sporodochia.
P = Pionnotes.
\+ = moderate, present.
++ = abundant, marked.

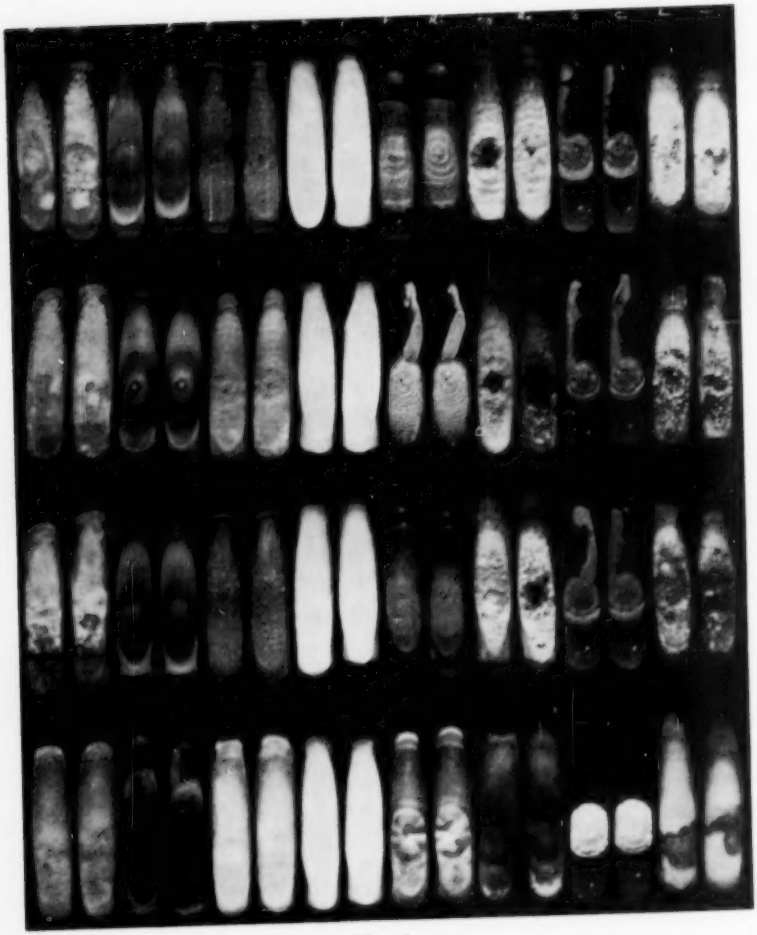

FIG. 1.

FIG. 1. Comparative cultural appearances of single clones of 8 *Fusarium* species after 30 days' growth under different environmental conditions, on potato dextrose agar. Top row, series 3; second row, series 4; third row, series 5; bottom row, series 6 (see page 460 and Table I). In pairs, in each row, left to right are *F. moniliforme*, *F. solani*, *F. oxysporum*, *F. tricinctum*, *F. rigidiuscula*, *F. roseum*, *F. episphaeria*, and *F. lateritium*. With fluctuating temperature (series 3), or fluctuating light (series 4), zonation is apparent in *F. solani* and *F. rigidiuscula*. *F. oxysporum* shows zonation in series 4. *Gibberella perithecia* were formed on *F. roseum* cultures in series 3, 4 and 5. There is no zonation in any culture in series 5 and 6. All cultures in series 6 (continuous darkness, continuous light)

It is of special interest that there was no zonation in any of the species under continuous light or continuous darkness with continuous temperature.

The development of pigment in relation to light and temperature varied in different species. As compared with cultures exposed to light, those in the dark showed more conspicuous pigmentation in *F. solani* (olive green), *F. rigidiuscula* (rose), and *F. roseum* (rose). On the other hand, cultures of *F. moniliforme*, *F. oxysporum*, *F. tricinctum* and *F. episphaeria* developed pigment only when exposed to light; cultures of *F. episphaeria*, different shades of orange, the others a pinkish tint. The cultures in the dark remained pure white. Although *F. lateritium* resembled *F. moniliforme* and other species in the development of the pinkish tint, bluish patches were present in cultures grown in the light and in the dark.

A wide divergence in sporulation was observed in the various species under different environmental conditions. *F. solani* showed abundant sporulation under all conditions studied. However, in the outdoor cultures it had a very poor growth of white mycelium and no sporodochia. Although the characteristic green growth with profuse sporulation was observed in the high temperature ranges of 25°–30° C, these cultures showed patch mutants of small round areas of white mycelium. Evidently, the high temperature of 25°–30° C in the chamber was too high a range for normal growth while the outdoor temperatures were too low for growth and sporulation.

Longer duration of light, higher temperature or large fluctuations in temperature range seemed to favor the pionnotal type of sporulation in *F. moniliforme* and *F. oxysporum*. Outside temperatures at the time of the experiment were found too low for the normal growth of both these species. It was learned from the preliminary experiment that both species produced sporodochia under conditions of fluctuating light and a constant temperature of 25° C. However, *F. moniliforme* had developed large granules in its spores while *F. oxysporum* under the same conditions showed no such abnormality. It was also observed that spore abnormalities in the nature of germ-tube anastomoses, or wavy margins, or large granules inside, were of common occurrence in cultures of both species grown under conditions that favored the pionnotal type of growth. The chamber with fluctuating light and constant tem-

show abundant mycelial development and no sporodochia nor pionnotes except slightly, in the center of the colony of *F. solani*. Differences in pigmentation (not evident in black and white photograph) are indicated in *F. episphaeria*, where the colonies in series 3, 4 and 5 are colored, while those in series 6 are pure white.

perature of 20° C provided an environment favorable for normal development. Nevertheless, cultures in the room (about 23° C) were more typical of the natural clone of both species.

F. rigidiuscula and *F. episphaeria* sporulated under all conditions except in the series in the dark, but the spores produced under most of these conditions showed abnormalities such as poor septation or the presence of large granules. Fluctuating light (12–12) and fluctuating temperature of 25°–30° C seemed favorable for growth and sporulation in both these species although the extent of growth shown by *F. episphaeria* was very small as compared with all the other species. Guttation water was a common feature in *F. episphaeria* while in *F. rigidiuscula* it was produced only in the cultures grown in fluctuating light (12–12) and a constant temperature of 20° C (and accounts for the "running" of the cultures of these fungi, seen in FIG. 1).

F. tricinctum, *F. roseum* and *F. lateritium* failed to produce sporodochia under any of the cultural conditions tried in this experiment, although the outdoor conditions in November had produced a favorable effect on the sporulation of these species on potato-dextrose agar and pea straw agar. It was observed that *F. roseum* developed perithecia under various conditions, the cultures exposed to fluctuating light and continuous temperature of 20° C producing them in abundance (FIG. 1). Sclerotial structures were prominent in *F. lateritium*. A few sclerotia were also produced in the *F. roseum* cultures, mostly in contact with glass. Under all conditions, *F. tricinctum* produced no reproductive structure other than the microconidia.

DISCUSSION

The importance of both light and temperature in relation to sporulation of fungi has long been recognized, but it is only recently that emphasis has been placed on a diurnal fluctuation in these factors for normal growth and fruiting. Houston and Oswald (**5**) found that an outdoor environment with 14 to 15 hours of daylight and average maximum and minimum temperatures of 26.8° and 8.2° C, respectively, was most favorable for sporulation in *Helminthosporium gramineum*. The beneficial effect of alternating light and darkness on the production of perithecia and of asexual spores in *Diaporthe phaseolorum* var. *batatatis* was demonstrated by Timnick et al. (**17**). Previous studies on *Fusarium* species in this laboratory have shown the favorable influence of a natural environment on sporulation. The results reported here confirm these findings and emphasize the different diurnal requirements of the different species.

The optimum conditions for so-called typical *in vitro* growth varies with the species of *Fusarium*. Fluctuating light (12–12) and a fluctuating temperature of 20°–23° C appeared to offer the requirements for normal development of *F. moniliforme*. Similarly, fluctuating light and a temperature varying from 20°–25° C seemed favorable for *F. oxysporum* and *F. solani*, although the latter species could adjust itself to a wider fluctuation in temperature. The conditions for normal growth and sporulation of *F. rigidiuscula* and *F. episphaeria* are probably 25°–30° C temperature and fluctuating light (12–12). On the other hand, *F. roseum* seems to require fluctuating light and temperatures never higher than 20° C for sporulation. *F. tricinctum* and *F. lateritium* failed to sporulate under any of the environmental conditions afforded in this experiment. It is reasonable to assume that these species also require as low temperatures as *F. roseum*.

The results of the preliminary experiment had shown normal growth and sporulation of several species in outdoor cultures exposed to diurnal conditions existing in Berkeley during November. However, the outdoor environment in January seemed unfavorable for all the species except *F. tricinctum*, *F. roseum*, and *F. lateritium*, all of which developed abundant aerial mycelium but no sporodochia. This evidently points to the influence of fluctuations in the diurnal as well as the seasonal environment on growth of fusaria in culture.

SUMMARY

Single spore cultures of eight of the nine species of *Fusarium* (new species concept) were grown on potato-dextrose agar under different environmental conditions. Their comparative development under fluctuating light and fluctuating temperature, fluctuating light and continuous temperature, continuous light and fluctuating temperature, continuous light and continuous temperature, and continuous darkness and continuous temperature was studied.

F. solani, *F. oxysporum*, *F. rigidiuscula* and *F. roseum* showed pronounced zonation in cultures grown under conditions of fluctuating light or fluctuating temperature.

There was a marked difference in pigmentation in cultures grown in the dark and in the light. *F. rigidiuscula* and *F. roseum* developed more conspicuous pigmentation in the dark as compared with cultures in the light. In all the other species, pigment was developed only in cultures exposed to light.

Cultures grown in complete darkness and constant temperature of

20° C failed to produce sporodochia or pionnotes in all species except *F. solani*. Diurnal fluctuations favored sporulation. However, wide fluctuations in temperature and long duration of light produced spore abnormalities. The optimum requirements for the so-called typical *in vitro* development of the different species appeared to be diurnal fluctuation in light and fluctuating temperatures of 20°–23° C for *F. moniliforme*, 20°–25° C for *F. oxysporum* and *F. solani*, 25°–30° C for *F. rigidiuscula* and *F. episphaeria* and temperatures fluctuating within narrow limits but never higher than 20° C for *F. roseum*, *F. tricinctum* and *F. lateritium*. These optima for sporulation are not to be confused with those for most rapid radial growth of mycelium.

Since only one clone of each of the eight species was used in these experiments, and since each of these species is known to be composed of many genetically different clones, it must be borne in mind that quite different results could result from a different choice of clones. However, since the clones used here were representative of those commonly occurring in nature, it is suggested that the indications of preferred environments shown by them may apply in general to recently cultured isolates of these species.

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THE INFLUENCE OF ACTIDIONE ON WOOD-STAINING FUNGI [1]

CHARLES L. FERGUS [2]

(WITH 1 FIGURE)

In many sections of the nation during certain seasons, lumber must be dipped in stain-preventive chemicals to reduce and prevent heavy degrade due to unsightly discolorations (2) caused by fungi. Where logs are not immediately sawed into lumber the same chemicals may be sprayed on the ends and bark-skinned areas of freshly cut logs (1). There are many proprietary compounds available on the market for stain control. These contain ethyl mercuric phosphate or chlorinated phenols which are toxic to fungi at low concentrations. However, *Ascocybe grovesii* Wells, the cause of "brown surface stain" or "brick-red stain" of conifers, is reported by Wells (3) to be unaffected by Permatox, which contains sodium pentachlorophenate.

Whiffen (4, 5) has shown that the antibiotic cycloheximide (Actidione) is inhibitory to a number of yeasts that cause disease in animals, and to many fungus pathogens of plants. The possibility of using Actidione as a stain preventive was investigated by observing its *in vitro* effect on various stain fungi, including *Ascocybe grovesii*. Its effectiveness was compared to pentachlorophenol.

MATERIALS AND METHODS

The basic medium consisted of 1.5 gm Difco malt extract and 1.5 gm agar per 100 ml of solution. The medium was autoclaved, cooled to 50° C, and sufficient quantities of the chemicals were added to produce the desired concentrations. This method of addition was used to elimi-

[1] Contribution No. 201 of the Department of Botany and Plant Pathology. Authorized for publication November 1, 1955, as paper No. 2023 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] The author wishes to express his gratitude to Mr. Ross W. Davidson, Division of Forest Pathology, U. S. D. A., Dr. J. L. Lowe, Syracuse University, and Miss Doreen E. Wells, Canadian Dept. of Agriculture, for supplying the fungus cultures used in this investigation. Appreciation is also expressed to the Upjohn Company, Kalamazoo, Michigan, who through the courtesy of Dr. William Klomparens furnished the crystalline Actidione used.

TABLE I

THE *in vitro* INFLUENCE OF ACTIDIONE UPON CERTAIN WOOD-STAINING FUNGI RELATIVELY INSENSITIVE TO 100 MICROGRAMS PER ML

Organism	Percent inhibition of growth	Organism	Percent inhibition of growth
Cadophora pini	0	*Ophiostoma ips*	0
Cytospora pini	10	*O. majus*	10
Endoconidiophora fimbriata	0	*O. multiannulatum*	4
Graphium rigidum	17	*O. picea*	0
Leptographium engelmanni	18	*O. pilifera*	10
L. lundbergii	0	*O. pini*	0
Ophiostoma canum	16	*O. pluriannulata*	10
O. coerulea	11	*O. ulmi*	17
		Sclerophoma entoxyli	53

nate any possible reduction of activity by autoclaving. Aqueous suspensions of spores of the test fungi were obtained from 14-day-old malt extract agar slant cultures. One drop of the spore suspension was pipetted onto the center of each petri dish containing 20 ml of the test media. The tests were replicated four times.

The effect of the chemicals was evaluated by measuring the diameter of the mycelial colony after 6, 8, 10 and 14 days' incubation at 24° C, since the fungi varied in their growth rates. Two diameter measurements were made to obtain an average of the largest and smallest diam-

TABLE II

THE *in vitro* INFLUENCE OF ACTIDIONE UPON CERTAIN WOOD-STAINING FUNGI SENSITIVE TO THE ANTIBIOTIC

Organism	Lowest conc.[a] completely inhibiting growth	Greatest conc.[a] allowing growth	Percent inhibition at greatest conc. allowing growth
Ascocybe grovesii (D)[b]	0.1	0.01	70
A. grovesii (C)[c]	1.0	0.1	80
Cadophora brunnescens	100[d]	10	75
Curvularia geniculata	100	10	53
Endoconidiophora moniliformis	10	1	87
Hormiscium gelatinosum	10	1	75
Ophiostoma adiposa	10	1	52

[a] Micrograms per ml.

[b] Culture obtained from Davidson. Isolated from *Picea sitchensis* in Colorado, 1944.

[c] Type culture obtained from Wells. Isolated from *Pinus strobus* in Ontario, Canada, 1952.

[d] Spores germinated but no spread from inoculum drop area occurred. See FIG. 1.

eters of asymmetrical colonies. The average was then compared, in percent, to diameter increase on malt extract agar which contained no added chemicals. Concentrations of Actidione tested were 100, 10, 1, 0.1, 0.01 and 0.001 micrograms per ml of agar. Concentrations of

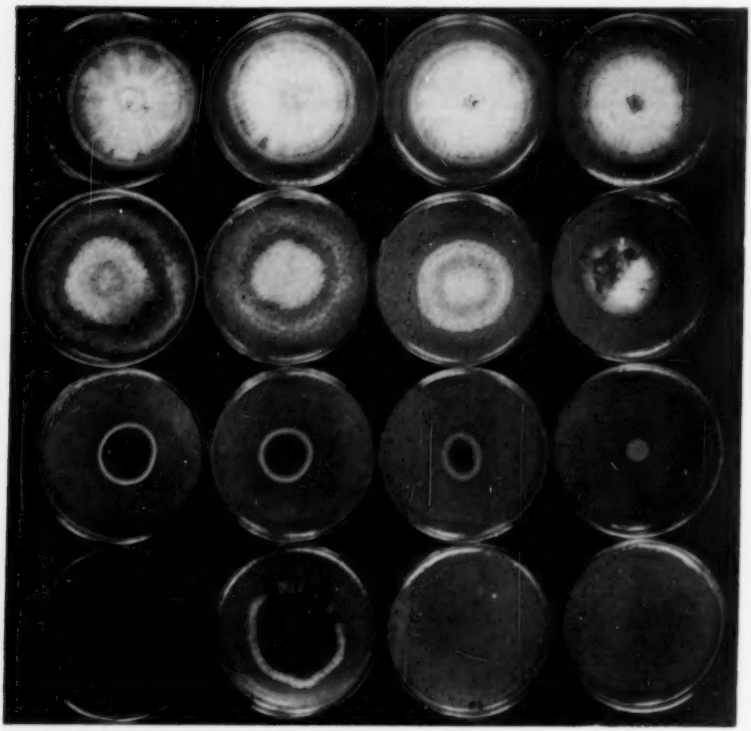

FIG. 1. The growth of four wood stain fungi in different concentrations of Actidione; from the left, 0, 1, 10, and 100 micrograms per ml. From the top: *Ophiostoma coerulea, Sclerophoma entoxyli, Cadophora brunnescens,* and *Ophiostoma adiposa.*

pentachlorophenol [3] tested were 2, 1, 0.5, 0.25, and 0.01 percent by weight of the active ingredient. The "Penta" was distributed well in the agar by thorough swirling in the flask prior to pouring and swirling the petri dishes after pouring.

[3] A commercial preparation by Chapman Co., called "Penta," containing 5 percent pentachlorophenol in hydrocarbon base, was used.

EXPERIMENTAL RESULTS

A considerable difference in sensitivity to Actidione was noted among the fungi tested. Those relatively insensitive to the antibiotic are listed in TABLE I.

The growth of some of these was measurably reduced; while that of others was not inhibited at all. Certain fungi were relatively sensitive to Actidione. They are listed in TABLE II. In addition, the greatest concentration allowing growth and the percent inhibition of growth at that concentration are also presented. *A. grovesii* was extremely sensitive to low concentrations of the antibiotic. A difference in sensitivity between two geographic isolates was observed. The reaction of four fungi are illustrated in FIG. 1.

TABLE III

THE *in vitro* INFLUENCE OF PENTACHLOROPHENOL [a]

Organism	Lowest conc.[b] completely inhibiting growth	Greatest conc.[b] allowing growth	Percent inhibition at greatest conc. allowing growth
Ascocybe grovesii (D)	Not tested	2	75
A. grovesii (C)	Not tested	2	70
Ophiostoma picea	0.1	0.01	68

[a] See footnote 3.
[b] Percent by weight.

The growth of *A. grovesii* in media containing Actidione was studied in more detail to determine whether the fungus would develop resistance to the antibiotic by successive transfers from media containing small amounts of Actidione (0.01 micrograms per ml) to media containing various higher concentrations of the chemical. No increase in resistance to Actidione was observed in the two isolates after four such transfers.

Pentachlorophenol was relatively non-toxic to *A. grovesii* when compared with its toxicity to *Ophiostoma picea*, as shown in TABLE III.

A small difference in tolerance to pentachlorophenol by the two geographical isolates of *A. grovesii* was observed. The growth made at the greater concentrations (0.5, 1 and 2 percent) was quite different from the type of growth made at the lower concentrations, being yeast-like at the higher, and mycelial at the lower concentrations.

DISCUSSION AND SUMMARY

Actidione had only a slightly inhibitory effect on the majority of the wood-staining fungi tested. Large differences in tolerance to the anti-

biotic among species of the genus *Ophiostoma* were noted. In addition, differences in tolerance were observed between two geographic isolates of *A. grovesii*, the Canadian isolate being less sensitive. It was also slightly less sensitive to pentachlorophenol than was the Colorado isolate. *Ophiostoma picea* proved to be much more sensitive to pentachlorophenol than was *A. grovesii*. Mr. E. A. Atwell, of the Canadian Forest Products Laboratory, was quoted by Wells [4] as stating that ". . . in the laboratory the fungus grows and fruits readily on white pine sapwood dipped for 10 seconds . . . in a solution of Permatox containing 10 lbs per 100 gallons of water." Although the effect of the antibiotic was tested *in vitro* and not on white pine sapwood the results obtained indicate the possibility of controlling brick-red stain caused by *A. grovesii* with Actidione.

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ANTIFUNGAL PROPERTIES OF TETRAZOLIUM COMPOUNDS [1]

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(WITH 1 FIGURE)

Although tetrazolium salts have been known for more than 60 years (**22**), the appreciation of their biological usefulness originated only in 1939 when they were first employed to determine seed viability (**15**). Because of their ease of reduction from soluble colorless compounds to insoluble pigmented formazans (FIG. 1), tetrazolium salts serve as convenient indicators of dehydrogenase activity (**10**, **22**). Primarily for this reason, these compounds are finding increasing application in studies on the dehydrogenases of microbial, plant, and animal tissues. They have also been adapted for histochemical techniques, bioautography, isolated enzyme systems, tissue culture, and insect studies.

Such investigations with a wide range of tetrazolium derivatives have established their general toxicity for microbial, higher plant, and metazoan cells. Other reports show that they are readily reduced by bacteria, on which they exert various effects (**1**, **8**, **12**, **24**, **26**), by pleuropneumonia-like organisms (**23**), mycobacteria (**4**, **9**, **11**, **27**), streptomycetes (**7**), and fungi (**3**, **6**, **13**, **16**). The evidence implicating dehydrogenase enzymes as the sites of tetrazolium inhibition (**2**, **9**, **14**, **21**, **22**) and the wide distribution of these electron transport systems among microorganisms, including fungi (**5**), suggested that certain animal pathogens in this group may possibly be controlled by tetrazolium salts. Although these agents are too toxic for systemic administration, they might nevertheless prove useful for topical treatment of dermatophytic infections where high concentrations can be applied locally. For these reasons, the present studies on antimicrobial properties of some tetrazolium derivatives were undertaken with yeasts and filamentous fungi.

MATERIALS AND METHODS

Seven fungi pathogenic to man were selected as test organisms. For comparison, two yeasts and two filamentous plant pathogens were

[1] The tetrazolium salts employed in these studies were kindly supplied by the Synthetical Laboratories, 5558 Ardmore Avenue, Chicago, Illinois.

included in studies on fungistasis. These organisms were maintained and tested at 25–28° C on the following tap water medium adjusted with NaOH to pH 6.8–7.0: KH_2PO_4 0.5 gm, $MgSO_4 \cdot 7H_2O$ 0.2 gm, $FeCl_3 \cdot 6H_2O$ 0.01 gm, $CaCO_3$ 0.01 gm (stock solution acidified with minimal HCl to dissolve), glucose 10.0 gm, molasses 2.5 gm, peptone 5.0 gm, and agar 15.0 gm per liter. Because of photochemical reactivity of certain tetrazolium salts, incubation was carried out under the uniform and continuous lighting conditions described elsewhere (**17**).

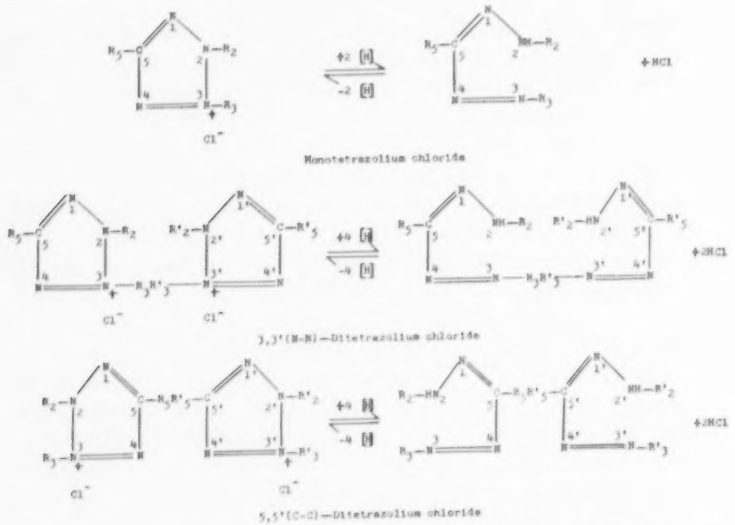

FIG. 1. Structural formulae and reactions of soluble, colorless, oxidized tetrazolium chlorides to form insoluble, pigmented, reduced formazans.

Fungistatic action was measured by a modified paper disc technique (**20, 25**). Large batches of agar medium were homogeneously seeded with spores or cells of the fungi and dispensed 12 ml per petri dish. The inoculum used for this purpose was the minimal amount of finely divided cell material (scraped from an agar slant into 5 ml of sterile tap water) that allowed uniform growth in the poured plates. Paper discs (13 mm in diameter) were each impregnated with three drops of test solution and then at once firmly appressed onto the agar surface. This procedure requires relatively small amounts of materials which need not be sterile, and is also convenient for mass testing. Plates were observed 24, 48, and 72 hours after addition of the discs, but only the

final 3-day readings are recorded in TABLES II and III. For determining fungicidal properties, dilute suspensions of cell material in the agar-free medium were prepared from slant cultures of the organisms. One ml portions of these suspensions were added to tubes containing one ml volumes of sintered glass-filtered tetrazolium solutions with and without 2% glucose. The final concentration of sugar after this 1:1

TABLE I

CHEMICAL COMPOSITION AND CONCENTRATION OF TETRAZOLIUM DERIVATIVES TESTED

Compound No.	Chemical composition*			Concentration, %	Ethanol, % by volume	pH	Turbidity of final preparation
	R_1 (and $R_3R'_3$)	R_2 (and R'_2)	R_3 (and R'_3)				
Monotetrazolium chlorides							
1	Phenyl	Phenyl	Phenyl	1.0	0	6.9**	None
2	Methyl	Phenyl	Phenyl	9.4	0	3.2	None
3	Dimethoxy-phenyl	Phenyl	Phenyl	3.2	0	3.3	None
4	*o*-Tolyl	Phenyl	Phenyl	1.0	13.4	7.1**	Heavy
5	*m*-Tolyl	Phenyl	Phenyl	1.0	0	7.1**	None
6	α-Naphthyl	Phenyl	Phenyl	1.0	6.7	6.8**	Slight
7	β-Naphthyl	Phenyl	Phenyl	1.0	13.4	6.8**	Slight
8	*o*-Biphenyl	Phenyl	Phenyl	1.0	13.4	7.2**	None
9	*m*-Biphenyl	Phenyl	Phenyl	1.0	0	3.0	None
10	*p*-Biphenyl	Phenyl	Phenyl	0.7	0	4.7	None
11	*o*-Biphenyl	*m*-Nitrophenyl	Phenyl	0.85	0	3.6	None
12	*p*-Biphenyl	*m*-Nitrophenyl	Phenyl	1.0	13.4	7.1**	Heavy
3,3′(N – N)—Ditetrazolium chlorides							
13	4,4′-Biphenylene	*m*-Nitrophenyl	Phenyl	1.0	13.4	7.0**	Heavy
14	4,4′-Biphenylene	Phenyl	Phenyl	1.0	13.4	7.0**	Slight
15	4,4′-Methylene-biphenylene	Phenyl	Phenyl	1.0	0	3.0	None
16	*o*-Dianisidyl	Phenyl	Phenyl	1.0	13.4	7.2**	Heavy

* Positions of R and R' substituents on compounds are indicated in FIG. 1.
** These solutions were adjusted to *p*H 6.8–7.2 with NaOH, and C_2H_5OH was then added to increase solubility except for compounds 1 and 5.

dilution was therefore 1%. After 6, 12, 29, and 48 hours incubation, a 10 mm wide bent wire rod was used to streak agar plates from each tube and the relative growth which resulted was estimated. In these fungicidal tests, the fungi were exposed to tetrazolium concentrations that were half the strength of the original stock solutions (TABLE I).

The chemical composition of the mono- and 3,3′(N-N)-ditetrazolium salts tested and the solutions employed are given in TABLE I. With

four exceptions, these were distilled water preparations at the 1% concentration that preliminary tests indicated to be most suitable for the fungistatic test procedure. The methyl and dimethoxyphenyl monotetrazolium chlorides, which were among the most soluble salts, were employed at 9.4% and 3.2%, respectively; compounds 10 and 11 were tested at somewhat less than 1%. Since pH can affect dissociation and influence the ability of tetrazolium cations to penetrate cell membranes, two different reactions were tried. Seven of the monotetrazolium and three of the ditetrazolium salts were neutralized with NaOH. Ethanol (95%) was then added in the amounts shown in TABLE I to insure solubility of the sodium salts. No significant differences in biological reactivity were observed whether a solution was adjusted to pH 6.8–7.2 or was used in the untreated acidic condition. Of the 16 preparations, three exhibited slight turbidity while four contained appreciable amounts of finely suspended material.

To evaluate antifungal activity accurately for those preparations neutralized with NaOH, controls for pH and chloride ions were necessary. For this purpose, portions of uninoculated media were adjusted with HCl to various pH levels going down as far as the most strongly acidic tetrazolium solution. In both the fungistatic and fungicidal tests, suitable experiments were routinely carried out to determine whether NaCl up to 1% and alcohol *per se* would inhibit growth. These preliminary tests showed that the low pH of the unadjusted solutions, the chloride ion, and the maximum 13.4% alcohol (TABLE I) exerted no appreciable effects of their own. In the absence of the tetrazolium cations, there was no demonstrable inhibition when low pH, NaCl and alcohol were tested alone or combined.

RESULTS

The data in TABLE II resulted from an experiment carried out with seven fungi pathogenic to man. These findings clearly show that *Sporotrichum schenkii,* which was inhibited by 15 of the 16 tetrazolium derivatives, was sensitive to the greatest number of compounds but not necessarily to any given compound. *Microsporum audouini,* on the other hand, was susceptible to the fewest compounds; its development was affected by only six tetrazolium salts. It is of particular interest that inhibition of growth and reduction of tetrazolium to formazan were not invariably associated. Clear zones in which the yeast *Candida albicans* failed to multiply were observed with 12 compounds, but pigmented peripheral zones where the tetrazolium salts had been reduced developed for 14 solutions. In addition to *C. albicans,* some fungi also converted

certain compounds which did not prevent growth into the colored formazans. On the other hand, many tetrazoliums inhibited growth without being reduced, as shown especially for *Epidermophyton floccosum*.

The yeasts and plant-parasitic fungi in TABLE III were selected to represent different organisms for comparison with the seven human pathogens listed in TABLE II. As shown in TABLE II, 72 out of 112

TABLE II

EFFECT OF TETRAZOLIUM COMPOUNDS* ON FUNGI PATHOGENIC TO MAN**

Compound No.	*Candida albicans*		*Epidermophyton floccosum*		*Microsporum audouini*		*Microsporum canis*		*Sporotrichum schenkii*		*Trychophyton mentagrophytes*		*Trychophyton rubrum*	
	I	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R*	*I*	*R*
Monotetrazolium chlorides														
1	25	+	0		0		20		28		0		0	
2	25	+	22		0		46		23		40		32	
3	25	+	42		25		38		38		26		23	
4	24	+	0		0		18		36		0		0	
5	24		0		0		25		33		0		0	
6	21	+	0		0	+	32		33		0		0	
7	23	+	18		21		22		40		32		16	
8	0	+	27		20		28		29		23		36	
9	18	+	16		0	+	29	+	29	+	15		0	
10	20	+	27	+	25	+	23	+	26	+	18	+	25	+
11	17	+	23		20		0		21		16	+	19	
12	0	+	0		0		0	+	14	+	0		0	
3,3′(N – N)—Ditetrazolium chlorides														
13	0	+	0		0		0		0		0		0	
14	16	+	0		0		18		19		16		0	
15	19	+	28		30		28		21		21		24	
16	0		26		0		0		15		16		42	

* See TABLE I for composition of compounds.

** *I* = inhibition in mm diameter of zone with no growth; zero indicates no zone surrounding the 13 mm diameter disc. *R* = reduction or formation of colored formazan surrounding inhibition zone.

(64.2%) of the tests showed inhibition; while 27 (24.1%) developed zones in which formazans had been produced. However, in TABLE III, 86% (55 out of 64) of the total tests were characterized by zones devoid of fungus growth, and 89.1% (57 of the 64) were associated with reduction of the tetrazolium. The seven particular human pathogens which were tested thus appeared to be more resistant to growth-inhibiting properties of these compounds and less capable of reducing them as

compared with the four organisms reported in TABLE III. However, a far greater number of species would have to be tested to determine whether pathogenic fungi as a group are more resistant to tetrazolium salts than are fungi non-pathogenic to man. It is also of interest that *C. albicans* converted a greater number of tetrazoliums to formazans

TABLE III

EFFECT OF TETRAZOLIUM COMPOUNDS* ON FUNGI NON-PATHOGENIC TO MAN**

Compound No.	*Saccharomyces cerevisiae*		*Hansenula saturnus*		*Ceratostomella ulmi*		*Fusarium moniliforme*	
	I	*R*	*I*	*R*	*I*	*R*	*I*	*R*
Monotetrazolium chlorides								
1	35	+	21	+	34		0	+
2	40	+	33	+	54		0	+
3	26	+	37		50		18	+
4	32	+	22	+	35		0	+
5	32	+	26	+	34		0	+
6	28	+	30	+	37	+	17	+
7	27	+	28	+	36	+	17	+
8	17	+	32	+	40	+	23	+
9	43	+	22	+	49	+	0	+
10	24	+	29	+	44	+	17	+
11	20	+	29	+	40	+	13	+
12	0	+	20	+	27	+	16	+
3,3(N – N)—Ditetrazolium chlorides								
13	0		17	+	25	+	16	+
14	20	+	19	+	29	+	19	+
15	22	+	27	+	44	+	17	+
16	0	+	0	+	26	+	17	+

* See TABLE I for composition of compounds.

** *I* = inhibition in mm diameter of zone with no growth; zero indicates no zone surrounding the 13 mm diameter disc. *R* = reduction or formation of colored formazan surrounding inhibition zone.

than did the six pathogenic molds (TABLE II). Among the non-pathogenic yeasts and fungi listed in TABLE III, formazan production was more uniformly distributed.

In order to determine whether growth inhibition resulted from fungistatic or fungicidal action, experiments were carried out with monotetrazolium compounds 2 and 10 and the ditetrazolium No. 15 against *M. canis, T. mentagrophytes, T. rubrum,* and *S. schenkii.* The results of this study showed that in both the presence and absence of glucose, all four pathogens were killed within 48 hours. Sugar was added as a hydrogen-donor in half the tests since transformation of the

tetrazolium salts to their corresponding formazans by an exogenous reducing agent might conceivably "detoxify" the system, *i.e.*, prevent interference with internal dehydrogenases. Stoichiometrically, the 1% glucose contained enough hydrogen for complete reduction of the three tetrazolium derivatives 2, 10, and 15, which were present at half the concentrations shown in TABLE I because of the 1:1 dilution of stock solutions involved in the procedure for determining fungicidal action. The lack of protection by glucose in the fungicidal tests and the independence of tetrazolium inhibition and reduction in the fungistatic tests indicate that tetrazolium action involves more than simple interference with dehydrogenase and hydrogen transport systems.

DISCUSSION

Inhibition of growth by tetrazolium compounds appears to be more selective for the seven human pathogenic fungi than for the four fungi non-infectious to man, but many more species would have to be studied before drawing any general conclusions. Nevertheless, the observations with the eleven organisms employed seem to indicate the reverse of what Fults *et al.* (**7**) reported for parasitic and non-parasitic strains of the plant pathogenic *Actinomyces scabies*. It remains to be determined whether the selective action exerted by these substances is as highly specific as the antifungal properties characteristic of antibiotic agents of microbial origin (**18, 20**). The rather widespread spectrum of fungistasis together with the fungicidal behavior of three tetrazolium salts suggests these compounds be considered for external treatment trials of dermatophytic infections.

Unfortunately, the relationships between molecular structure and biological activity do not permit generalizations. For example, of the two m-nitrophenyl monotetrazolium chlorides, the *o*-biphenyl derivative was more inhibitive to the pathogenic fungi, while the p-biphenyl compound was reduced by more organisms than were inhibited (TABLE II). However, both forms were broadly fungistatic when tested with the four cultures in TABLE III, all of which reduced the *o*- as well as the p-biphenyl salt. Of the 4,4′-biphenylene ditetrazoliums, the m-nitrophenyl was less reactive than the phenyl derivative. At the time of these investigations, only mono- and 3,3′(N-N)-ditetrazolium salts were available. Since then, the synthesis of various 5,5′(C-C)-ditetrazoliums has provided isomers of the 3,3′(N-N) compounds which may give interesting comparative results. As shown in FIG. 1, the 5,5′(C-C) forms are linked through the sole carbon atoms of the heterocyclic tetrazoles, whereas in the 3,3′(N-N) structures the linkage involves the

No. 3 nitrogen atoms. However, sterically the derivatives are quite different. The No. 2 and 3 nitrogen atoms which are involved in reductive transformation to the formazan are "peripherally" located in the 5,5′(C-C)-ditetrazolium but more "centrally" arranged in the 3,3′(N-N) form. This in itself indicates that differences in biological activity may be expected.

Aside from such considerations, the results obtained in these studies suggest the possibility of developing differential techniques for isolating pure cultures of human pathogens by supplementing suitable media with tetrazolium compounds at concentrations which would not affect growth of the particular organism sought. Under such circumstances, the toxicity of these salts for most bacteria and non-pathogenic fungi would be advantageous. Whether strain specificity would be encountered in response to tetrazolium products is not yet known. If the strains of a particular species behave differently vis-à-vis these compounds, this might be a serious obstacle to the exploitation of tetrazolium salts for isolation purposes. But at the same time, such variation may be useful in characterizing strains. In connection with this, it may prove interesting to investigate a large number of independently isolated cultures for each of several species to determine their susceptibility to tetrazolium derivatives as growth inhibitors and, likewise, their ability to reduce these compounds.

This might also entail corollary studies on (a) variation in biological response to tetrazolium salts depending on physiological stage of the organism, (b) development of strains characterized by tetrazolium-resistance, and (c) the ability of these substances to effect variations such as the "duel phenomenon" in *Trichophyton mentagrophytes* (**19**). The application of tetrazolium salts to studies on the intermediary metabolism and general biochemistry of pathogenic fungi may also merit serious consideration.

The results in TABLE II reveal that fungi may react to tetrazolium salts in two different ways: (a) they may be sensitive or resistant to these compounds as growth inhibitors, and (b) they may or may not produce the colored formazans by reduction. Moreover, such responses apparently occur independently of one another. Growth inhibition and development of formazan are compatible with the concept of tetrazolium interference with dehydrogenase systems. But inhibition of growth without formazan production, as actually occurred in an appreciable number of cases, suggests a different mode of antifungal action. Unfortunately, the available data do not indicate what other mechanism(s) may be involved.

With respect to formazan production, the reaction of various tetrazolium compounds in lightness and darkness may give significant differences. With certain of these materials, both enzymatic and non-enzymatic reductions occur only in the presence of light, while in the dark pigments do not form except via non-enzymatic reduction in strongly alkaline solution. On the other hand, light may convert certain tetrazolium derivatives into so-called "photo-compounds" which are unable to undergo reduction to the formazans. Moreover, formazans may suffer photochemical decomposition as a result of an autocatalytic reaction initiated by the "photo-compounds" which act as catalysts. Consequently, as pointed out by Jambor (**10**), the effects of tetrazolium salts in biological systems may be more complex than heretofore appreciated.

SUMMARY

The objective of these studies was to obtain information about the antifungal properties of tetrazolium derivatives. For this purpose, twelve monotetrazolium and four 3,3′(N-N)-ditetrazolium chlorides were used in fungistatic tests with seven organisms pathogenic to man and with four cultures non-parasitic for animals. The results showed that the tetrazolium derivatives were generally more inhibitive, and more selectively so, for the particular human pathogens than for the four other fungi that were selected, but these numbers of organisms are not adequate to draw generalizations about the two groups. Three of the 16 compounds were found to be fungicidal. These observations suggest that tetrazolium salts, despite their general toxicity to higher animals, may justify consideration as agents for the external treatment of dermatophytic infections.

The unexpected finding that inhibition of growth and reduction of tetrazoliums to formazans are independent phenomena is discussed in terms of the possible mode of action of these substances. It is concluded that the interrelationships between tetrazolium compounds and biological systems are more complex than has been assumed, especially because of non-enzymatic photochemical reductions apparently not yet fully appreciated. Possible applications of tetrazolium derivatives for isolation, diagnostic work, and physiological studies of pathogenic fungi are pointed out.

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RECOVERY OF THE COMPONENT STRAINS FROM DIKARYOTIC MYCELIA [1]

PHILIP G. MILES AND JOHN R. RAPER

(WITH 4 FIGURES)

In the dikaryon of the Basidiomycetes two unfused, compatible, functionally haploid nuclei coexist in the same cell. This association of the nuclei is perpetuated in each subsequent cell generation by conjugate nuclear division, which is generally associated with a particular type of hyphal septation resulting in the familiar clamp connection. Mycelium composed of cells of this type is known as secondary or dikaryotic mycelium. Cells containing dikaryons are genetically the equivalent of cells containing diploid nuclei. The dikaryotic condition, a genetically balanced system, is extremely stable, with remarkably few deviations from the regular pattern of conjugate division and clamp connection formation. When separation of the nuclear components of the dikaryon can be accomplished, however, the system offers a unique opportunity for studying nucleo-cytoplasmic relations.

Two different means have been employed for separating the nuclear components of the dikaryon. One method makes use of monokaryotic oidia arising from the dikaryotic mycelium. This has been used by Aschan (**1**) but is, of course, restricted to those species such as *Collybia velutipes* that normally dedikaryotize in this manner.

The other and more generally applicable method involves a microsurgical operation on hyphae during clamp formation. This was employed by Harder (**6**) and more recently by Fries and Aschan (**5**), and Papazian (**10**). The terminal and hook cells are punctured with a micromanipulator at a stage in development of the clamp prior to fusion of the hook with the penultimate cell. The penultimate cell is uninucleate at this stage, and following the microsurgical operation, this cell is transferred to nutrient medium, where it grows into a homokaryotic mycelium. This procedure is laborious and operational errors frequently result in failure to recover homokaryons. For example, Papazian (**10**) recovered only 31 homokaryons out of 130 operated cells.

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In the course of investigations on the restriction of radial growth of colonies of *Schizophyllum*, it was observed that dikaryotic mycelium grown in the presence of sodium taurocholate produced sectors composed of unclamped hyphae. Subsequent tests in liquid medium containing sodium taurocholate indicated that homokaryotic mycelia of both nuclear types could be recovered readily.

METHODS

The medium used contained mineral salts, glucose, and peptone to which 0.15% sodium taurocholate, or 0.12% cholic acid, was added.[2] The dikaryon was allowed to grow in liquid medium for 2 to 3 weeks or longer. The culture was then macerated in a Waring blendor, aliquots of the macerate inoculated into flasks of agar cooled to 45° C, shaken, and poured into Petri dishes.[3]

In an attempt to minimize subjective selection of colonies to be examined in determining the effectiveness of a substance as a dedikaryotizing agent, a pie-shaped wedge was marked on the bottom of the plate, and starting at the apex all colonies within this wedge were isolated until fifty had been obtained. Following incubation for a few days, the isolates were examined microscopically for the presence or absence of clamp connections, and all unclamped mycelia were saved for later mating type determinations and morphological characterization. The following definitions have been employed in order to evaluate the results obtained as to the effectiveness of dedikaryotization of the various test compounds.

$$\text{Dikaryon Stability Coefficient} = \frac{\text{No. dikaryons}}{\text{No. isolates}}$$

$$\text{"Splitting" Coefficient (Dedikaryotization Effectiveness)} = 100\left[1 - \frac{\text{Dikaryon Stability Coefficient for Treatment}}{\text{Dikaryon Stability Coefficient for Control}}\right]$$

[2] Other substances have also been tested. Desoxycholic acid, which is closely related to cholic acid and thus also to sodium taurocholate, has been used as well as Tergitol-7. The latter was used because it had shown a restrictive effect on radial growth of *Schizophyllum*, and because it is a surface active agent. The bile acids and salts used possess a somewhat similar property in that they facilitate the solution of many compounds.

[3] It is desirable to restrict the radial growth of the colonies, but so far all compounds tested which performed this function when added to the medium have had certain undesirable features which argue against their use. Rose bengal is one of the more successful compounds in this respect and has been used in a concentration of 0.003% in some of the experiments described here.

expressed as a percent. The values of the "splitting" coefficient can range from 100 downward to minus values. A "splitting" coefficient of 20 or higher has generally been found to permit the recovery of both nuclear components in a sample of fifty isolations.

RESULTS

Dikaryotic mycelia of 19 different species of Basidiomycetes have been used in this study. The present report is based upon a study of 13 of these, but in five cases more than one strain of the species was investigated. In some species the treatment described above has resulted in marked separation of the dikaryon into its component homokaryotic parts. These species are placed in two categories, the first of which (TABLE I) contains those stocks in which oidia have been observed. Only in the cases of *Collybia velutipes* and *Corticium effuscatum* have the oidia (or in the case of *Corticium*, the secondary spores) been observed on the dikaryotic mycelia. Where oidia are formed, one would expect to recover homokaryotic mycelia without treatment, but the high values obtained for the "splitting" coefficients indicate significant increases in dedikaryotization in the presence of the test substances.

TABLE I

STOCKS IN WHICH OIDIA WERE OBSERVED

Dikaryon	Dikaryon stability coefficient			"Splitting" coefficient		M.T.'s recovered
	Control	Cholic acid	Na-tauro-cholate	Cholic acid	Na-tauro-cholate	
Collybia velutipes (Åschan)*	47/50		1/47		97.8	2
Collybia velutipes (Q.M.C.)	48/48	0/29	8/11	100	(17)	—
Corticium effuscatum (Nobles)	82/88	2/49	0/83	95.6	100	2
Lenzites betulina, No. 1	40/50		12/48		69	2
Lenzites betulina, No. 2	42/49		17/49		59.5	2
Poronidulus conchifer	49/50		2/46		95.6	2

* In this and the other tables the name in parentheses indicates the source of the culture. Where no name is given the culture is from the collection maintained at Harvard by J. R. R. The intials Q.M.C. indicate that the culture was obtained from the stock collection of the Quartermaster Corps Research and Development Center, Natick, Massachusetts.

In the second category are placed those species in which oidia have not been observed and in which the dikaryons have been "split" by the treatment. In some cases homokaryons have been isolated from the

TABLE II

STOCKS IN WHICH OIDIA WERE NOT OBSERVED

Dikaryon	Dikaryon stability coefficient			"Splitting" coefficient		M.T.'s recovered
	Control	Cholic acid	Na-taurocholate	Cholic acid	Na-taurocholate	
Favolus alveolaris	36/42		5/41		86	2
Pleurotus ostreatus	31/50		5/49		83.5	2
Pleurotus ostreatus (Q.M.C.)	24/47	0/27	0/41	100	100	—
Polyporus betulinus	43/45		0/44		100	—
Polyporus versicolor (Q.M.C.)	18/50	0/46	2/49	100	89	2

controls. (The recovery of unclamped mycelia from blendor macerates of dikaryons has also been observed by Raper, **11**, and Papazian, **10**.) The effectiveness of the test compounds in "splitting" the dikaryons, however, is quite marked (TABLE II).

The dikaryons of some stocks were unaffected by the treatment (TABLE III). In general, replication of the results of the treatment with a given species was good. A marked difference was obtained, however, in the results of two trials on the effect of sodium taurocholate on *Cyathus stercoreus*, for which there is as yet no adequate explanation.

Marked stock differences have been observed with the species *Schizophyllum commune* in respect to the effect of the bile acids on "splitting" the dikaryon. Some of the dikaryons are extremely stable in the pres-

TABLE III

STOCKS IN WHICH TREATMENT WAS INEFFECTIVE

Dikaryon	Dikaryon stability coefficient			"Splitting" coefficient		M.T.'s recovered
	Control	Cholic acid	Na-taurocholate	Cholic acid	Na-taurocholate	
Cyathus poeppigii, aberrant (Brodie)	95/95		99/99		0	0
Cyathus poeppigii (Brodie)	99/99		86/88		2	1
Cyathus stercoreus (Brodie)	50/50		49/50		2	1
Ptychogaster rubescens (Q.M.C.)	36/38		1/23		95.4	—
Stereum purpureum (Q.M.C.)	28/28		31/31		0	0
	49/49	95/96		1		1

ence of the bile acid while others have fairly high "splitting" coefficients (TABLE IV).

Phase microscopic observations have indicated a possible mechanism by which this splitting of the dikaryon may take place. Hyphae have been frequently observed in which the hook cell failed to fuse with the penultimate cell, and the bulge in the penultimate cell where the hook cell would ordinarily fuse has grown out as a branch. Thus instead of a clamp or pseudo-clamp two branches with simple septa arise and cross one another giving a "bow-tie" appearance (FIGS. 1, 4). The terminal cell of the main hypha is binucleate and all derived cells are uninucleate (FIG. 2a, b). Frequently near the periphery of a colony growing in the presence of cholic acid, for example, only hyphae composed of uninucleate cells with simple septa can be seen (FIG. 3).

TABLE IV

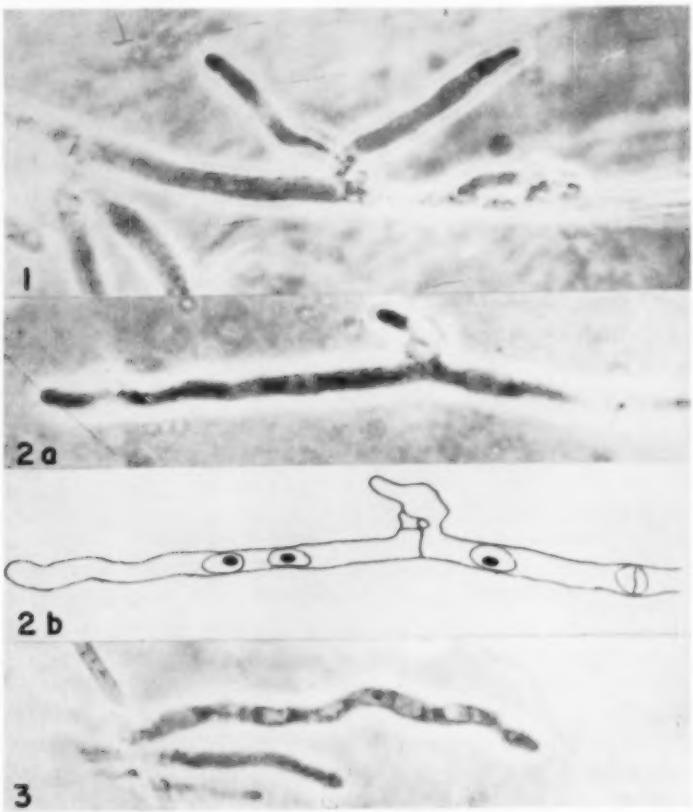
STOCKS OF SCHIZOPHYLLUM COMMUNE IN WHICH A VARIABLE RESPONSE TO TREATMENT WITH BILE ACIDS IS EXHIBITED

Stock	Dikaryon stability coefficient			"Splitting" coefficient		M.T.'s recovered
	Control	Cholic acid	Na-tauro-cholate	Cholic acid	Na-tauro-cholate	
Fresh Pond	50/50		48/48		0	0
Magnolia	36/47		27/50		30	2
New Guinea (Q.M.C.)	25/39	1/73	47/48	99.8	−53	2
Walden II	50/50		50/50		0	0

Another possible mechanism is the laying down of simple septa between members of a dikaryon. Subsequent branching would then result in hyphae having uninucleate cells. Such a progression has not been observed microscopically, but large, swollen cells have frequently been observed in hyphae having mainly simple septa, with occasional clamp connections.

Several species have been grown on agar containing the test substances, the mycelia examined under the phase contrast microscope, and the observations compared with those obtained in control cultures. In all cases where phase microscopic examinations have been made, one of the test substances produced a "splitting" effect, as evidenced by the presence of simple septa, pseudoclamps, or the "bow-tie" character (TABLE V). It thus seems probable that a very high percentage of species can be dedikaryotized by bile acids once proper concentrations,

incubation periods, and selective methods are worked out. Thus, although the universal applicability of this method in recovering homokaryons from dikaryons has not been established, it is indicated that a majority of species treated will respond.



FIGS. 1–3. 1. Dikaryon of *Lenzites betulina* growing on medium containing cholic acid and showing a "bow-tie." 2, a. Phase photomicrograph of a dikaryon of *Lenzites betulina* growing on medium containing cholic acid showing a young "bow-tie" in which the terminal cell is binucleate and the penultimate cell is uninucleate. 2, b. Drawing of hypha shown in 2a indicating the positions of the nuclei and septa. 3. Hypha from the periphery of colony of *Lenzites betulina* growing on medium containing cholic acid showing uninucleate terminal cell and simple septum. Cells in the older portions of the colony were dikaryotic and bore clamp connections. All approximately × 750.

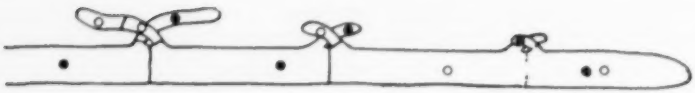

1. Subterminal cells uninucleate
Terminal cell binucleate
2. Nuclei in opposite wings of each "bow-tie" derived from different component strains

FIG. 4. Diagram showing development of "bow-tie" and nuclear composition of cells.

DISCUSSION

The study of the interactions of the dikaryotic components with special emphasis placed upon the influence of dikaryotic cytoplasm upon the resulting homokaryotic mycelia has, in the past, been the main

TABLE V

PHASE MICROSCOPE OBSERVATIONS OF DIKARYOTIC MYCELIA GROWING ON MEDIA SUPPLEMENTED WITH A BILE ACID

Species	Age in days at time of observation	Control	Cholic acid	Na-taurocholate
Schizophyllum commune (Q.M.C.)	12	True clamps	Simple septa, Pseudoclamps	Atypical
Schizophyllum commune (Lilly 577)	16	True clamps	True clamps, Bow-ties	True clamps
Schizophyllum commune, Walden I	16	True clamps	Simple septa, Bow-ties, True clamps	True clamps, Bow-ties
Schizophyllum commune, Walden II	16	True clamps	True clamps, Simple septa	True clamps
Corticium effuscatum (Nobles)	13	True clamps	Atypical Simple septa (growth slight)	True clamps (growth slight)
Cyathus peoppigii (Brodie)	15	True clamps	Simple septa, True clamps	True clamps
Lenzites betulina	12	True clamps	True clamps, Pseudoclamps, Simple septa,	True clamps
Pleurotus ostreatus	14	True clamps	True clamps, Simple septa, Pseudoclamps	True clamps, Simple septa, Pseudoclamps
Polyporus versicolor (Q.M.C.)	13	True clamps	Simple septa	Simple septa

Note: It has not been possible to observe the nuclear condition in all cases since at the age when they were examined most of the hyphae were highly vacuolate, rendering phase microscopic examination of the nuclei very difficult. From the successful observations, however, it is probably safe to assume that (1) where simple septa were seen, the cells were uninucleate, (2) where pseudoclamps and "bow-ties" were seen the cells were uninucleate except for the tip cell, and (3) where true clamps were seen the cells were regularly dikaryotic. More than one classification given for a treatment indicates that the colony had sectors in which the different types of septation could be observed.

reason for separating the components of the dikaryon. Harder (6), Fries and Aschan (5), Aschan (1), and Papazian (10) have all suggested that a cytoplasmic effect is probably the best explanation for the aberrancies in homokaryotic mycelia isolated from dikaryotic mycelia. It might be possible to use the technique described in this paper for such studies, but, as with the other techniques employed, precautions should be taken to use only acceptable genetic procedures. This is essential since it is possible to isolate morphologically distinct mycelia arising as sectors which could have their origin in genic mutations. For example, homokaryotic isolates of the same mating type but of distinct morphologies were obtained from a dikaryon of *Corticium effuscatum* treated with sodium taurocholate. These aberrancies were practically identical with mycelia obtained by subculturing from sectors arising in an unclamped, isolated homokaryon.

Other studies can likewise be greatly facilitated by the use of the "splitting" technique. Investigations in somatic recombination could be carried out by growing dikaryons composed of genetically identified biochemical and morphological mutant strains in the presence of cholic acid or sodium taurocholate and then using selective media to recover the recombinants. A preliminary trial using this technique, however, was unsuccessful.

It is frequently desirable to know the nuclear composition of a fruit body obtained from nature. One stock of *Schizophyllum* which we are studying is of especial interest because its progeny segregate for an uncommon biochemical deficiency or complex of deficiencies, as well as for a minimum of four morphological variants. Furthermore, the progeny exhibit very marked differences in fruit body forming capacity when mated with compatible strains. Possession of the dikaryotic components would give valuable information, but as yet it has been possible to recover only one of the nuclear components. This is probably because of selection against a biochemically deficient and morphologically aberrant strain.

Finally, we suggest that the technique should prove useful to those interested in identification of wood-rotting Basidiomycetes in cases where it has not been possible to obtain single spore isolates of the fungus for interfertility tests, or to induce the clamped mycelium to form fruit bodies. The homokaryotic component strains of an unknown dikaryon would give compatible strains which, if they form clamp connections when mated with one another or with the original dikaryon, would be capable of accepting nuclei from known homokaryotic or dikaryotic testers of the same species. Such interfertility tests have

been used in the past for establishing the identity of a culture with that of a known species. Mounce and Macrae (7) determined that cultures from different fruit bodies of *Fomes roseus* were interfertile but that lack of interfertility existed between these isolates and those from fruit bodies of *Fomes subroseus,* the isolates of which were interfertile when mated. Nobles (8) used interfertility tests to demonstrate the incorrectness of a previous identification of a wood-rotting organism and to establish its current identity. This was done by showing lack of interfertility with cultures of *Trametes serialis,* which had earlier been thought to be the organism causing the decay, and by showing complete fertility with cultures of an organism described as *Poria microspora.*

In addition to interfertility tests, hyphal fusions have been used to establish the specific identity of two cultures (Davidson *et al.,* 3; Brodie, 2). This method has certain disadvantages, since anastomoses frequently occur among hyphae from the same inoculum, and much intermingling of hyphae may occur before fusions are formed between hyphae from the two inocula being tested. Tracing the hyphae back from a fusion to both original inocula may be a laborious task and frequently cannot be accomplished with absolute certainty.

The bile acid method for bringing about dedikaryotization should provide a useful extension of interfertility tests in the identification of Basidiomycetes. The following procedure is suggested:

(1) Homokaryotic mycelia are obtained from the unknown dikaryon by the procedure given in this paper.

(2) The recovered homokaryon is then mated with the original dikaryon from which it was recovered to determine by a Buller phenomenon type of reaction whether it is a unilateral or a bilateral mater. If it is a unilateral dikaryotizer—that is, if it does not accept nuclei from the dikaryon—then the utility of the isolate is limited to those cases where it can be mated with a homokaryon known to be a bilateral mater. It has no value in a Buller phenomenon type of reaction since it has been shown by Terra (12) that homokaryons which behave unilaterally when mated with compatible homokaryons also behave unilaterally when mated with dikaryons possessing compatible nuclei.

(3) The recovered homokaryon is then mated with identified dikaryons which have been selected as possibly being of the same species as the unknown on the basis of cultural criteria obtained for the unknown dikaryon according to the methods used by Nobles (9) or Davidson *et al.* (4). If one is dealing with a tetrapolar species the chance of obtaining a negative reaction when mating two homokaryons of different stocks due to the presence of common "alleles" is around two

percent according to Whitehouse (**13**). When one is dealing with a Buller phenomenon the only possibility for a negative reaction is when the homokaryon possesses an A "allele" present in one member of the dikaryon and a B "allele" present in the other nucleus of the dikaryon. This is the situation in which in some cases a positive reaction results even though only incompatible nuclei are involved and has become known as the illegitimate Buller reaction. This situation would be expected to arise in only 0.04 percent of the cases. Therefore, when dealing with a tetrapolar species with the unknown being capable of accepting nuclei (*i.e.* being a bilateral dikaryotizer), the homokaryon should become dikaryotized by a dikaryon of the same species in more than 99.9 percent of the cases in which the mating is made. If it is a question of a bipolar species, a bilateral homokaryon would be expected to become dikaryotized in a Buller phenomenon reaction in every case, provided the species are the same.

It is thus evident that the isolation of large numbers of homokaryotic components by means of the chemical splitting of the dikaryon makes feasible certain analyses and experimental studies which have, up to now, been impractical because of the time-consuming techniques required.

SUMMARY

Dikaryotic mycelia of many Basidiomycetes grown in the presence of sodium taurocholate or cholic acid are dedikaryotized into their component homokaryotic strains. Differences in the "splitting" of the dikaryon exist among various species and even between stocks of a given species. Phase microscopic observations indicate that the dedikaryotization takes place by a disruption of the clamp connection formation process.

The dedikaryotization technique described here should prove useful in the following studies: 1. the effect of dikaryotic cytoplasm upon homokaryotic mycelia; 2. somatic recombination; 3. identification of unknown dikaryons by interfertility tests.

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MARINE FUNGI. I. LEPTOSPHAERIA AND PLEOSPORA

T. W. JOHNSON, JR.[1]

(WITH 35 FIGURES)

The marine fungi have received scant attention in the past decade, despite the work of Barghoorn and Linder (**1944**) which opened an entirely new field of endeavor, both taxonomically and physiologically. Knowledge of marine fungi in general is extremely limited, judging from the more recent surveys of published literature on these organisms (Barghoorn and Linder, **1944**; Wolf and Wolf, **1947**; Zobell, **1946**).

Among the marine fungi first to receive attention were Ascomycetes occurring on remains of marine and brackish-water phanerogams, and on marine algae. Cooke and Plowright (Cooke, **1887**) described *Sphaeria maritima* (on *Juncus maritimus*), a species later transferred to *Leptosphaeria* by Saccardo (**1883**). *Leptosphaeria chondri* was described by Rostrup (**1889**) under the binomial *L. marina*. Rosenvinge (**1906**) renamed Rostrup's fungus *L. chondri*, since *L. marina* was preempted (Ellis and Everhart, **1885**). *Leptosphaeria discors* was described by Saccardo and Ellis (**1883**) under the binomial *Metasphaeria discors*. Descriptions of *L. marina*, *L. spartinae*, and *L. sticta* were published by Ellis and Everhart in **1885**. *Leptosphaeria albopunctata* and *L. incarcerata* are also known from marine habitats. The most recent addition to the marine species of *Leptosphaeria* is that of Linder (Barghoorn and Linder, **1944**), *L. orae-maris*, inhabiting submerged wood.

In the genus *Pleospora*, three marine representatives are known, two of which occur on marine algae. *Pleospora laminariana* was described by Sutherland (**1916**); *P. pelvetiae* by the same author in **1915**. The third species, *P. maritima*, was described by Rehm (Saccardo, **1899**, p. 600) on *Triglochin maritima*.

[1] I wish to express my sincere appreciation to Dr. C. G. Bookhout, Director, Duke University Marine Laboratory, and to Dr. A. Carter Broad, Resident Investigator, for making available the facilities of the Laboratory. Grateful appreciation is tendered the University Council on Research for funds to defray a portion of the expense of the investigation. Drs. Wm. H. Weston, J. N. Couch, L. E. Wehmeyer, L. S. Olive, E. K. Goldie-Smith, and Mr. S. P. Meyers contributed generously of their time in criticizing the manuscript.

Marine species of *Leptosphaeria* are not uncommon, either on submerged wood, or on decaying culms and leaves of marine phanerogams. In the case of the leptosphaerias, however, most species which are known are not illustrated, and, in some instances, are incompletely diagnosed. A survey of leptosphaerias and pleosporas in marine habitats is lacking; hence individual collections are not easily identified. The present paper is an attempt to redescribe certain of the species of *Leptosphaeria* as they are known to occur in Beaufort Inlet, Beaufort, North Carolina, on submerged wood, and on culms of *Spartina alterniflora* and *Juncus maritimus*. In addition, a new marine species of *Pleospora* is described.

LEPTOSPHAERIA DISCORS (Sacc. & Ellis) Sacc. & Ellis, Mich. 2: 567. 1879.

Metasphaeria discors Sacc. & Ellis, *In* Saccardo, Syll. Fung. 2: 173. 1883.

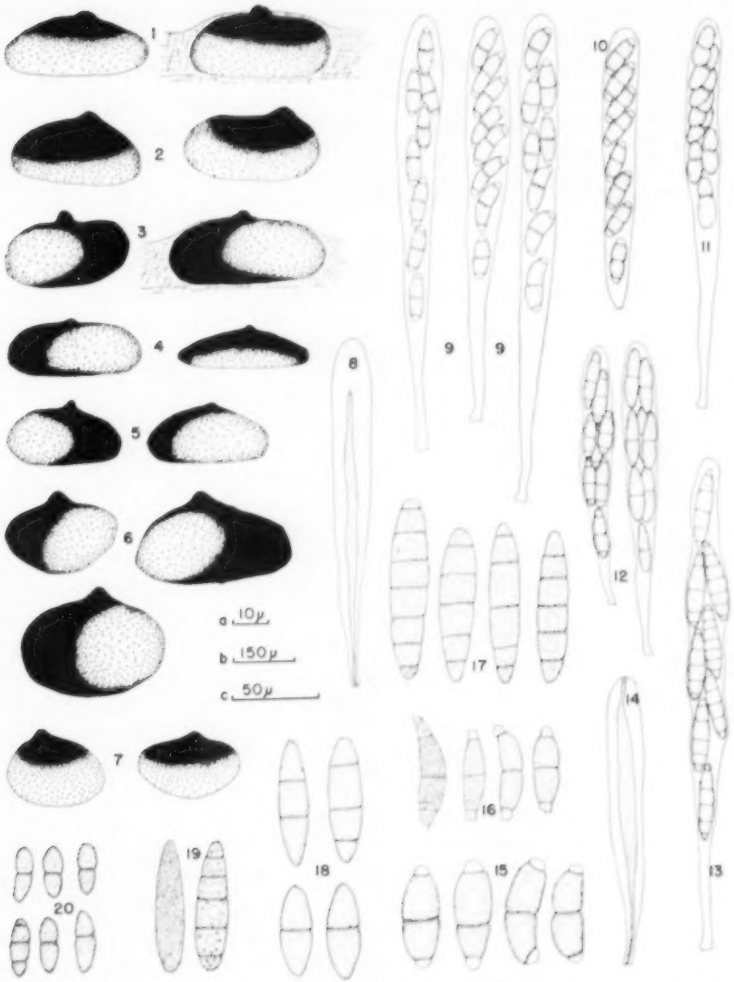
FIGS. 1, 2, 9, 10, 14–16

Perithecia scattered or gregarious, subglobose to ellipsoid, innate, ostiolate, the ostioles black, short, papilliform, exserted above the substratum, black above, fading to brown below, 200–388 μ in diameter, 105–183 μ high. Asci few, clavate or cyindric, 8-spored, 160–288 × 24–30 μ, thick-walled, becoming thin-walled at maturity. Ascospores broadly fusoid, straight or curved, brown, 3-septate, constricted at the septa, end cells rounded, hyaline, uniseriate to biseriate, 28–33 × 9.5–13 μ. Paraphyses filiform, simple or sparingly-branched, apices globose, acute, or cylindric.

Substratum: driftwood, and culms of *Spartina alterniflora*.

The Beaufort collections of *Leptosphaeria discors* differ principally in ascus size from the collections described by Ellis and Everhart (1892, p. 373). Saccardo and Ellis report an ascus size of 120 × 20 μ. There seems no way of accounting for this discrepancy, but the difference does not justify describing another species.

On *Spartina alterniflora*, the perithecia of *Leptosphaeria discors* protrude only at the short ostiole; on wood, the ascocarps are completely imbedded except for the ostiolar pore. On either substrate the innate fruiting bodies form slight pustules. The ascospores are rather characteristic. When mature, the end cells are hyaline, the remaining cells are brown, and each has a single guttule (FIG. 15). Within the basal portion of the perithecium the asci are few and always sharply curved. Thus, the upper portion of the ascus lies roughly parallel with the long axis of the perithecial base.



FIGS. 1–20. *Leptosphaeria*. 1, 2, 9, 10, 14, 15, 16. *L. discors*. 1, 2, Perithecia. 9, 10. Asci and ascospores. 14. Immature ascus. 15. Mature ascospores. 16. Stages in maturation of ascospores. 3–5, 8, 13, 17, 19. *L. albopunctata*. 3–5. Perithecia. 8. Immature ascus. 13. Ascus and ascospores. 17. 5- and 7-septate ascospores. 19. Stages in maturation of ascospores. 6, 12, 18. *L. marina*. 6. Perithecia. 12. Asci and ascospores. 18. Mature ascospores. 7, 11, 20. *L. orae-maris*. 7. Perithecia. 11. Ascus and ascospores. 20. Mature ascospores. Figs. 1–7, scale b; 8–10, 12–14, scale c; 11, 15–20, scale a.

Berlese (Ic. Fung. 2: 51. 1900) created the genus *Passeriniella* for leptosphaeriaceous fungi with 3-septate ascospores having hyaline end cells. If this genus is to be recognized, *L. discors* should be transferred. Judging from previous publications on leptosphaerias, Berlese's proposal has not been generally accepted.

LEPTOSPHAERIA MARINA Ellis & Everhart, Jour. Mycol. 1: 43. 1885.

FIGS. 6, 12, 18

Perithecia scattered, globose, subglobose, globose-conic, or globose-ellipsoid, innate, ostiolate, ostioles short, black, papilliform, exserted above the substratum, black throughout, 187–395 μ in diameter, 105–308 μ high. Asci few, clavate or cylindric, 8-spored, thick-walled, becoming thin-walled at maturity, 115–179 × 18–28 μ. Ascospores fusiform or clavate-fusiform, straight, yellow to yellow-brown, 1–3-septate, constricted at the septa, end cells rounded, 35–48 × 8–11 μ, uniseriate to partially triseriate. Paraphyses filiform, simple or branched, ends clavate or cylindric.

Substratum: culms of *Spartina alterniflora* and *Juncus maritimus*.

The ascospores of the Beaufort collections of *Leptosphaeria marina* are not as long, nor do the asci have as great a diameter as those described for the species by Ellis and Everhart (1892, p. 372). In other features the collections are similar.

Superficially, the perithecia of *Leptosphaeria marina* resemble those of *L. discors* (*cf.* FIGS. 1 and 6). The perithecia are larger than those of *L. discors*, however, and are coal-black throughout. In both *L. marina* and *L. discors*, the whitish ostiolar pore is prominently displayed, suggesting one of the diagnostic features of *L. albopunctata*. The ascospores of *L. marina* and *L. discors* are obviously different (*cf.* FIGS. 9, 10 and 18) in both size and septation. Except for spore size and perithecial color, *L. marina* is very close to *L. orae-maris*. The 3-septate spores of *L. marina* resemble those of *L. maritima* (*cf.* FIGS. 18 and 28). In the latter, however, the mature spores always have a gelatinous sheath (or gelatinized spore wall), while those of the former do not.

Leptosphaeria scirpi (*L. vagans* var. *scirpi*) seems indistinguishable from *L. marina*. From descriptions of the former, it appears that there is merely a host difference, hardly a significant diagnostic feature. Further studies of *L. scirpi* are needed to determine its relationship to *L. marina*.

LEPTOSPHAERIA ALBOPUNCTATA (West.) Saccardo, Syll. Fung. **2**: 72. 1883.

Sphaeria albopunctata West., Bull. Acad. Roy. Belg. Ser. II. **7**: 87. 1859.

Sphaeria incarcerata B. & C., Grevillea **4**: 152. 1876.

Leptosphaeria incarcerata (B. & C.) Sacc., Syll. Fung. **2**: 86. 1883.

Leptosphaeria sticta E. & E., Jour. Mycol. **1**: 43. 1885.

Leptosphaeria spartinae E. & E., Jour. Mycol. **1**: 43. 1885.

FIGS. 3–5, 8, 13, 17, 19

Perithecia scattered, subglobose, ovate, broadly ovoid, rarely broad-ellipsoid, innate, ostiolate, the ostioles short, black, papilliform, exserted above the substratum, black throughout, 217–390 μ in diameter, 161–255 μ high. Asci few, clavate or cylindric, 8-spored, thick-walled, becoming thin-walled at maturity, 135–293 × 11–30 μ. Ascospores fusiform or broad-fusiform, yellow, straight, 5–7-septate, slightly constricted at the septa, end cells rounded, each cell with a single guttule at maturity, uniseriate to biseriate, 30–55 × 10–15 μ. Paraphyses filiform, simple or sparingly branched, apices filiform, rarely clavate.

Substratum: culms of *Spartina alterniflora*, and rotting driftwood.

The lengthy synonymy indicates that *Leptosphaeria albopunctata* is a variable species, forms of which have been described as separate entities. *Leptosphaeria sticta* was reduced to synonymy by Ellis and Everhart (**1892**), and there is no doubt that the taxon is correctly relegated. In the writer's judgment, *L. incarcerata* and *L. spartinae* are also variants of *L. albopunctata*.

The distinctive feature of *Leptosphaeria albopunctata* is the punctiform ostiole which appears as a whitish or subhyaline dot. Judging from the description by Ellis and Everhart (**1892**, p. 375), the ostioles are reduced to mere ostiolar pores. One illustration of this species by Westendorp (**1859**, fig. 16) clearly shows a papilliform ostiole. Other species of *Leptosphaeria* collected on *Spartina* in the Beaufort Inlet region also show a whitish ostiolar pore; hence the characteristic hardly seems distinctive for *L. albopunctata* alone.

The principal differences between *Leptosphaeria albopunctata*, *L. incarcerata*, and *L. spartinae* seem to be merely those of ascus and ascospore size. *Leptosphaeria incarcerata* is very meagerly described, and, as Ellis and Everhart point out (**1892**, p. 374), ". . . this [*L. incarcerata*] cannot differ much from *L. albopunctata*. . . ." From the descriptive features which are known, *L. incarcerata* is distinctive only by reason of a "little clouded speck" above the perithecium, presumably the

ostiolar pore. This feature is not at all restricted to *L. incarcerata*. Berlese claims that the type of *L. incarcerata* has spores similar to those of *L. discors* both in septation and coloration. *Leptosphaeria incarcerata* is placed in *Passeriniella* by Berlese.

Upon first consideration, *Leptosphaeria spartinae* seems obviously distinct from *L. albopunctata*, especially in ascus and ascospore size. In the Carolina collections, however, these sizes are extremely variable. TABLE I illustrates the variability based on 10 of the 23 collections of perithecia referable to *L. albopunctata* and *L. spartinae*.

The data in TABLE I are indicative of a similarity between *Leptosphaeria albopunctata* and *L. spartinae*. Both species were described originally with 5-septate ascospores; in the Beaufort material, at least,

TABLE I

A COMPARISON OF 10 COLLECTIONS REPRESENTING L. ALBOPUNCTATA AND L. SPARTINAE

No.	Ascus size	Ascospore size	Number of septa
3	261–293×22–28μ	31–38×8–12μ	5
4	126–167×9–14μ	35–45×9–14μ	5–7
5	188–240×16–23μ	43–55×11–15μ	5
8	115–179×18–28μ	35–48×8–11μ	5–7
10	203–254×15–23μ	38–43×11–14μ	5–7
11	155–191×10–15μ	30–38×8–12μ	5–7
12	130–162×9–15μ	33–39×8–14μ	5
15	211–263×18–26μ	38–45×10–14μ	5–7
16	188–233×20–24μ	43–52×9–12μ	5
19	221–273×20–24μ	30–40×10–15μ	5–7

the spores are 5–7-septate. Other features such as color and shape of perithecia, and color of the ascospores are characteristics which both species have in common. Rather than consider the Beaufort collections as representatives of new varieties of *L. albopunctata* and *L. spartinae*, the writer prefers a grouping of the taxa into a single entity.

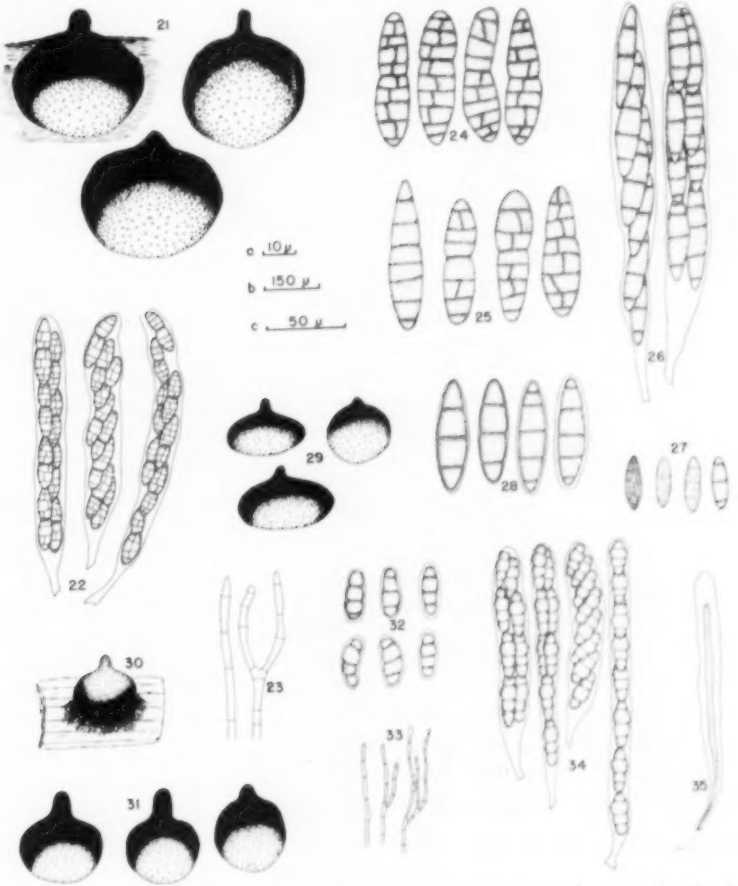
LEPTOSPHAERIA MARITIMA (Cke. & Plowr.) Saccardo, Syll. Fung. **2**: 73. 1883.

Sphaeria maritima Cke. & Plowr., *In* Cooke, Grevillea **5**: 120. 1877.

FIGS. 26–29

Perithecia scattered, globose, subglobose or broad-ellipsoid, innate, ostiolate, the ostioles short, black, papilliform, exserted above the substratum, black, 180–250 μ in diameter, 100–180 μ high. Asci few, clavate or cylindric, thick-walled, becoming thin-walled at maturity, 8-spored, 120–140 × 14–18 μ. Ascospores fusiform, yellow to yellow-brown,

straight, 3–5-septate, not constricted at the septa or inconspicuously so, end cells usually rounded, guttules lacking, gelatinous sheath present when mature, partially or completely biseriate, 32–40 × 8–12 μ. Paraphyses filiform, simple, apices filiform or apiculate.



FIGS. 21–35. *Pleospora* and *Leptosphaeria*. 21–25. *P. pelagica*. 21. Perithecia. 22. Asci and ascospores. 23. Paraphyses. 24, 25. 7- and 9-septate ascospores. 26–29. *L. maritima*. 26. Asci and ascospores. 27. Stages in maturation of ascospores. 28. Ascospores. 29. Perithecia. 30–35. *L. halima*. 30. Habit sketch of superficial perithecium. 31. Perithecia. 32. Mature ascospores. 33. Paraphyses. 34. Asci and ascospores. 35. Immature ascus. Figs. 21, 29–31, scale b; 22, 27, 33, scale c; 24–26, 28, 32, 34, 35, scale a.

Substratum: culms of *Spartina alterniflora* and *Juncus maritimus*.

Leptosphaeria maritima resembles the forms of *L. albopunctata* with 5-septate spores. Although the ascospores of some fruiting bodies of the latter species may be the same size as those of *L. maritima*, two differences are outstanding. In *L. maritima* the mature ascospores are surrounded by a gelatinous sheath (FIG. 28), while those of *albopunctata* are not; the latter produces prominently guttulate ascospores, whereas *L. maritima* does not. Linder reports *L. maritima* as having 5-septate ascospores, but the original description by Cooke and Plowright describes the ascospores as 3–5-septate, as the Beaufort collections show.

LEPTOSPHAERIA ORAE-MARIS Linder, *In* Barghoorn & Linder, Farlowia 1: 413. 1944.

FIGS. 7, 11, 20

Perithecia scattered, subglobose or broad-ovoid, innate, ostiolate, the ostioles short, black, papilliform, exserted above the substratum, black above, brown below, 160–255 μ in diameter, 125–135 μ high. Asci few, clavate or cylindric, thick-walled, becoming thin-walled at maturity, 8-spored, 98–141 × 8–12 μ. Ascospores broad-fusiform, brown, straight or curved, 1–3-septate, prominently constricted at middle septum, slightly constricted at other septa, in uniseptate spores, one cell usually large, end cells rounded, not conspicuously guttulate, uniseriate to biseriate, 18–25 × 5–8 μ. Paraphyses filiform, simple or sparingly branched, apices filiform, clavate, or apiculate.

Substratum: rotting driftwood and culms of *Spartina alterniflora*.

The two collections of this species differ slightly from the collection from California described by Linder. In general, the Beaufort material is slightly larger, the ascospores are definitely 1–3-septate, and not tardily 3-septate as Linder described. In size, the species suggests *Leptosphaeria discors*, but the ascospores are quite different (*cf.* FIGS. 15 and 20). This is apparently the first report of *L. orae-maris* from the East Coast, and from culms of *Spartina*.

Leptosphaeria halima sp. nov.

FIGS. 30–35

Peritheciis solitariis, globosis v. elongato-globosis, superficialibus v. innatis, ostiolatis, 114–216 μ diam., 180–252 μ altis. Ascis paucis, clavatis v. cylindricis, octosporis, 64–108 × 8–14 μ, saepissime 70–80 × 9–12 μ. Ascosporis fusiformibus, ovoideis v. cylindricis, flavido-brunneis, 3-septatis, gelatinosis, 1-seriatis v. 2-seriatis, 12–18 × 5–8 μ, saepissime 12–16 × 5–7 μ. Paraphysibus simplicis aut pauco-ramosis.

Perithecia scattered, globose or elongate-globose, superficial or partially innate, ostiolate, the ostioles variable in length, black, elongate-papilliform, black, 114–216 μ in diameter, 180–252 μ high. Asci few, clavate or cylindric, thick-walled, becoming thin-walled at maturity, 8-spored, 64–108 × 8–14 μ, predominantly 70–80 × 9–12 μ. Ascospores fusiform, ovoid or cylindric, yellow-brown when mature, straight or slightly curved, 3-septate, wall constricted at septa, guttules lacking, one cell usually large, uniseriate to biseriate, surrounded with a conspicuous gelatinous sheath when mature, 12–18 × 5–8 μ, predominantly 12–16 × 5–7 μ. Paraphyses few, simple or sparingly-branched, apices filiform or clavate.

On yellow poplar (*Liriodendron tulipfera*) veneer test panel, submerged off Shackleford Point near Beaufort, North Carolina, July 29, 1955, TYPE; decaying culms of *Spartina alterniflora*, Gallant Point, North Carolina, July 30, 1955.

Two collections of *Leptosphaeria halima* were made, one from brackish water along the Intracoastal Waterway, a second from test panels in open water. The ascospores of *L. halima* seem quite distinctive, and will immediately separate this species from other marine leptosphaerias. One cell of the 4-celled spore usually has a conspicuously greater diameter than the other cells (FIG. 32), suggesting a similar type spore in such species as *L. tenera*, *L. galiorum*, and *L. dryadis*, among others. The gelatinous sheath, ascospore size, and ascospore color distinguish *L. halima*, however,

Leptosphaeria halima differs from other known marine members of the genus in its partially or totally innate perithecia on herbaceous stems, but superficial ascocarps (FIG. 30) on wood. The gelatinous sheath is suggestive of *L. maritima*, but spore size as well as other characteristics of *L. halima* are very much different.

The new leptosphaeria suggests *L. typharum* in the subgenus *Sclerocleela* (Müller, **1950**, pp. 214, 219), but *L. typharum* has innate perithecia and considerably smaller asci. The two species are similar in ascospore shape. In the subgenus *Euleptosphaeria* (Müller, **1950**, p. 229), *L. dumentorum* is suggestive of the new species, but ascospore size and shape in *L. dumentorum* are different, and the perithecia are innate. Among the leptosphaerias with gelatinous ascospore walls, *L. poae* seems near *L. halima*. The spores of *L. poae* are hyaline. Of the foregoing previously described species of *Leptosphaeria*, none are known from marine habitats.

The reduction of two species of *Leptosphaeria* to synonymy and the designation of a new species in the same genus may seem inconsistent,

especially in a genus which presently has over 300 described species. *Leptosphaeria halima* is, however, clearly a marine form, and is very different from known marine representatives of the genus. For these reasons, the designation of the fungus as new seems warranted.

Pleospora pelagica sp. nov.

FIGS. 21–25

Peritheciis solitariis, globosis v. subglobosis, partim aut complete innatis, ostiolatis, nigris, 340–390 μ diam., 324–350 μ altis. Ascis paucis, cylindraceis v. clavatis, octosporis, 151–218 × 16–28 μ, saepissime 160–200 × 18–25 μ. Ascosporis fusiformibus, fusoideo-ellipticis v. clavato-ellipticis, aequilateralibus v. inaequilateralibus, muriformibus, 7–9-septatis, flavido-brunneis, partim aut complete 2-seriatis, 30–52 × 10–15 μ, saepissime 40–48 × 10–13 μ. Paraphysibus simplicis aut pauco-ramosis.

Perithecia scattered, globose to subglobose, partially to completely innate, ostiolate, the ostioles short, black, papilliform, exserted above the substratum, black, 340–390 μ in diameter, 324–350 μ high. Asci few, cylindric or slightly clavate, thin-walled at maturity, 8-spored, 151–218 × 16–28 μ, predominantly 160–200 × 18–25 μ. Ascospores fusiform, fusoid-ellipsoid or clavate-ellipsoid, usually inequilateral, symmetric or asymmetric, one end usually narrow and tapering, conspicuously constricted at one septum at or near the middle, the septum dark and prominent, usually muriform, with the end cells occasionally longitudinally septate, spores rarely without longitudinal septa, ends broadly rounded or acute, 7–9 transverse septa, yellow-brown, partially or entirely biseriate, 35–52 × 10–15 μ, predominantly 40–48 × 10–13 μ. Paraphyses few, simple or sparingly-branched, apices filiform, clavate, or apiculate.

On culms of *Spartina alterniflora*, Gallant Point, North Carolina, June 22, 1955, TYPE; on culms of *S. alterniflora*, Horse Island, near Taylor Creek, Beaufort, North Carolina, June 23, 1955.

Judging from recent papers by Wehmeyer (**1948**, **1949**), *Pleospora pelagica* belongs in the *herbarum* series in the genus, since the end cells of the ascospores occasionally have longitudinal septa. The spore form is characteristically that of the modified type, number 4, of Wehmeyer (**1948**, p. 272), in which the two halves of the spore are of unequal size (FIG. 24).

Pleospora pelagica differs from *P. pelvetiae* principally in size of asci and ascospores, those of *P. pelagica* being larger. Furthermore, the spores of *P. pelvetiae* are 7-septate, whereas the spores of the new fungus are 7–9-septate. *Pleospora laminariana* (Sutherland, **1916**, p. 260) has 5–7-septate ascospores, and smaller asci than those of *P. pelagica*.

Pleospora maritima Rehm was not found during the Beaufort study;

hence comparison of this species with *P. pelagica* must depend on published descriptions. Ascus length and perithecial size in *P. maritima* are very similar to those of *P. pelagica*. The ascospores of the new pleospora are nearly as long as those of *P. maritima*, but are much narrower.

Wehmeyer (**1948**, p. 281, fig. 42) illustrated spores from the type collection of *P. maritima*, and in comparing Wehmeyer's figures with the spores of *P. pelagica*, it is apparent that septation is markedly different. In Rehm's species, longitudinal septa are commonly 2–3 per cell, whereas in *P. pelagica* only one longitudinal septum is produced in any cell. The spore shape is noticeably different in the two species.

SUMMARY

Leptosphaeria discors, *L. albopunctata*, *L. marina*, *L. maritima*, and *L. orae-maris* are marine species known to occur in the coastal region near Beaufort, North Carolina. *Leptosphaeria halima*, occurring on submerged wood and on culms of *Spartina alterniflora*, is described as new. *Leptosphaeria incarcerata* and *L. spartinae* are considered synonymous with *L. albopunctata*. A new marine *Pleospora*, *P. pelagica*, is described from culms of *S. alterniflora*.

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DEVELOPMENT OF THE ASCOCARP OF ANTHRACOBIA MELALOMA [1]

MARTIN A. ROSINSKI [2]

(WITH 35 FIGURES)

Our knowledge of the developmental morphology of Operculate Discomycetes is mostly restricted to the ascogenous system. The sexual structures are conspicuous, and a large proportion of the work contributing to our knowledge of sexuality in Ascomycetes has been done on members of this group. However, emphasis was laid on nuclear behavior in the ascogenous system, and other aspects of development were mentioned only casually. Corner (**1929, 1930, 1931–1932**) recognized this fact, and his work, which involves a number of species, still stands as the best source of information we have on the development of the sterile portion of the ascocarp. Fitzpatrick's (**1917, 1918**) two papers on the development of the ascocarp and on sexuality in *Rhizina undulata* are also worth mentioning in this connection. They represent an attempt to do a reasonably complete job of following development through all phases.

A collection of *Anthracobia melaloma* (Alb. & Schw. *ex* Pers.) Boud. was made in the summer of 1953. This organism is not only a very convenient one to use for studying development, but it should also prove to be a good tool for studying the physiological factors involved in ascocarp development. Most important in this regard is that fruiting occurs readily on sterile agar media supplemented with the proper nutrients.

Anthracobia melaloma is a small cup-fungus which is found infrequently upon recently burned wood. Seaver (**1942**) includes it in his genus *Patella* which incorporates all "small" Operculate Discomycetes with hairs. It ranges in color from light buff to brownish, but the hairs are always dark brown.

In the present work, development has been followed in detail from the time of archicarp initiation to the point where the ascogenous hyphae

[1] A thesis presented to the Faculty of the Graduate School of Cornell University in partial completion of the requirements for the degree of Doctor of Philosophy, September, 1955.

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are giving rise to asci. There has been no effort made to study the cytology of the developing ascus. Some of this work has been done by Olive (**1950**).

Included also are a few experiments on genetical and environmental conditions which influence development of the ascocarp.

MATERIALS AND METHODS

SOURCE OF LIVING MATERIAL: Apothecia of *Anthracobia melaloma* were collected in 1953 by Professor Richard P. Korf, and this collection has provided most of the material for subsequent work. Cultures were obtained by inverting apothecia over sterile petri dishes containing sterile agar media and allowing the apothecia to shoot their spores onto the agar. The apothecia were inverted by fastening them to the underside of the top of the petri dish with vaseline jelly so that the hymenial surface faced downward.

A large percentage of the spores obtained in this manner germinated within 24 hours after landing on the agar surface. Single germinated spores were transferred to test tubes containing a sterile nutrient medium solidified with agar. Seven of these single spore cultures have been maintained up to the present time. Two replicates of each culture were made. All cultures have been stored under paraffin oil. One of the replicates was used in the regular routine of culture work and the other was maintained as a reserve, and was used when transfers were made.

The 7 single spore cultures obtained here could be divided into 2 groups on the basis of mating type, but they were all assigned consecutive numbers from 1 to 7. Numbers 4 and 6 represent one mating type and the remaining isolates represent the other mating type. Numbers 1 and 4 were used in most of the work.

The original collection from which the cultures were otained is in the possession of Professor Korf where it is being kept as part of his work on the flora of New York State. The number assigned to it is CUP *43752*.

CULTURE TECHNIQUES: The usual culture medium was 2% malt extract ("Difco") in distilled water solidified with 2% agar. This is the medium on which the stock cultures have been maintained and it was used in most of the experimental work. In a few instances, 2% agar in distilled water plus just a trace of malt extract or 2% agar in distilled water without nutrients were used. The last mentioned medium is the one which was used for inducing spore germination when cultures were first established.

All glassware (except test tubes) was sterilized in an oven heated to 175° F for 1 hour. Agar media and test tubes were sterilized in an autoclave at 15 pounds pressure for 20 minutes.

Where material was grown to be sectioned, a modification of Claussen's (**1912**) double petri dish technique was used. The inner dish (7 cm in diameter) was filled with malt agar, and a thin layer of water agar (2 mm deep) was added to the outer dish (14 cm in diameter). Mycelia of both mating types were inoculated at points near the center of the smaller dish and incubated at 24° C from 6 to 14 days.

Two types of slide culture techniques were employed to obtain whole mounts of young ascocarps. One of these was described by Riddell (**1950**). Using this type of slide culture, the only readily observable ascocarps were those which developed from hyphae growing on the glass. The other slide culture technique was that used by Shoemaker (**1955**). This type of slide culture permitted observation of colonies growing directly on agar. Malt agar was poured into the bottom of a petri dish containing 2 glass rods bearing a slide. The slide bore a thin layer of trace malt agar on its upper surface. Inocula from cultures 1 and 4 were placed in the petri dishes on either side of the culture slide. The two mycelia were allowed to grow from opposite sides and intermingle on the slide (FIG. 34). These cultures were usually left for 6 days before they were removed to be fixed and stained.

Observations on hyphal anastamoses were made using a modification of the Riddell slide culture technique. Two malt agar blocks were placed on opposite sides of a slide. Each of these blocks was inoculated with mycelium of a single mating type. A long cover slip was placed over the two blocks. The mycelium from each block grew out onto the glass of the slide and of the cover slip. After about 48 hours, intermingling of the two mycelia was observed, and the material was immediately fixed and stained.

FIXING, CUTTING, AND STAINING: For observing early developmental stages, stained whole mounts were prepared from the slide cultures mentioned above. Material obtained using the Riddell slide culture technique was usually mounted directly in acetocarmin. The acetocarmin was prepared according to the technique described by Johansen (**1940**), and served as the fixative, stain, and mounting medium. The slides were sealed with Waller's cement (**1949**) and were kept refrigerated over the period that they were needed for reference.

The Shoemaker slide cultures and the slide cultures prepared for observation of hyphal anastamoses were fixed in a solution consisting of 4 parts of 70% alcohol to 1 part of glacial acetic acid. They were

then stained in a solution consisting of 1 part of distilled water, 2 parts of glycerine, and 1 part of a saturated aqueous solution of erythrosin. The staining schedule followed was that described by Gwynne-Vaughan (**1937a**, pp. 366–367). The glycerine jelly was prepared according to the method described by Johansen (**1940**).

Apothecia embedded in paraffin were sectioned to observe the developmental sequence beyond the point where the sheath had enveloped the archicarp. Material was fixed at intervals varying from 2 to 8 hours. The fixative generally used was the weak chrome-acetic mixture described by Johansen (**1940**). Agar blocks averaging 1 cm square were cut out of the area in the double dishes where the water agar was poured. It was in this area that the apothecia were most abundant and where there was a minimum of aerial mycelium. Blocks bearing apothecia were placed in shell vials of fixative and were left for 24 hours. The fixed material was rinsed by covering the mouths of the vials with a layer of cheesecloth and placing them in a shallow, wide-mouthed vessel through which passed a gentle flow of tap water. Gentle washing was necessary to prevent the tiny apothecia from being washed off the agar blocks.

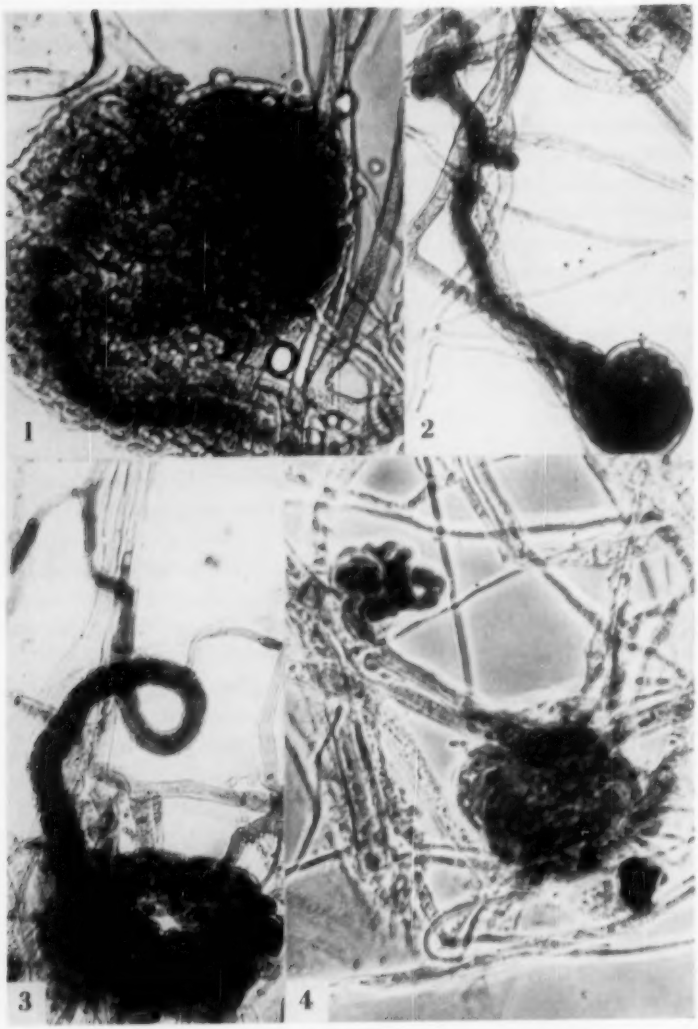
Subsequent dehydration and infiltration with paraffin was carried out using the tertiary butyl alcohol method as described by Johansen (**1940**). The agar blocks were trimmed of most of the excess agar before final embedding.

Sections were most commonly cut at 10 μ on a rotary microtome. These sections were too thick for careful cytological work, but they were of a convenient thickness for tracing hyphae.

Heidenhain's iron hematoxylin with erythrosin as a counterstain was used exclusively with sectioned material. The iron hematoxylin was prepared using methyl cellosolve as described by Johansen (**1940**), and his staining schedule was followed up to the point where the stained slides were immersed in 95% alcohol. After removal from the 95% alcohol, the slides were flooded with a carbolic acid-turpentine combination as prescribed by Durand (**1911**). The counterstain was then added and poured off after a few seconds. After 1 rinse in pure clove oil, the slides were placed in xylol (2 changes) and finally mounted in a synthetic resin ("Permount").

EXPERIMENTS AND OBSERVATIONS

THE ASCOGENOUS PORTION OF THE APOTHECIUM: Gwynne-Vaughan (**1937b**) described the young archicarp fairly well. Her page of illus-



FIGS. 1-4.

FIGS. 1-4. 1. Whole mount of archicarp prepared from Riddell slide culture and stained in acetocarmin. Photographed at ×600 and reproduced at ×300. Archicarp enclosed with ensheathing hyphae; the trichogyne is the darkly stained row of cells near the bottom of the photograph. 2, 3. Same as above but showing naked archicarps with differentiated ascogonial and trichogyne regions and branch-

trations showing the undifferentiated coil should suffice for a description of this phase of development.

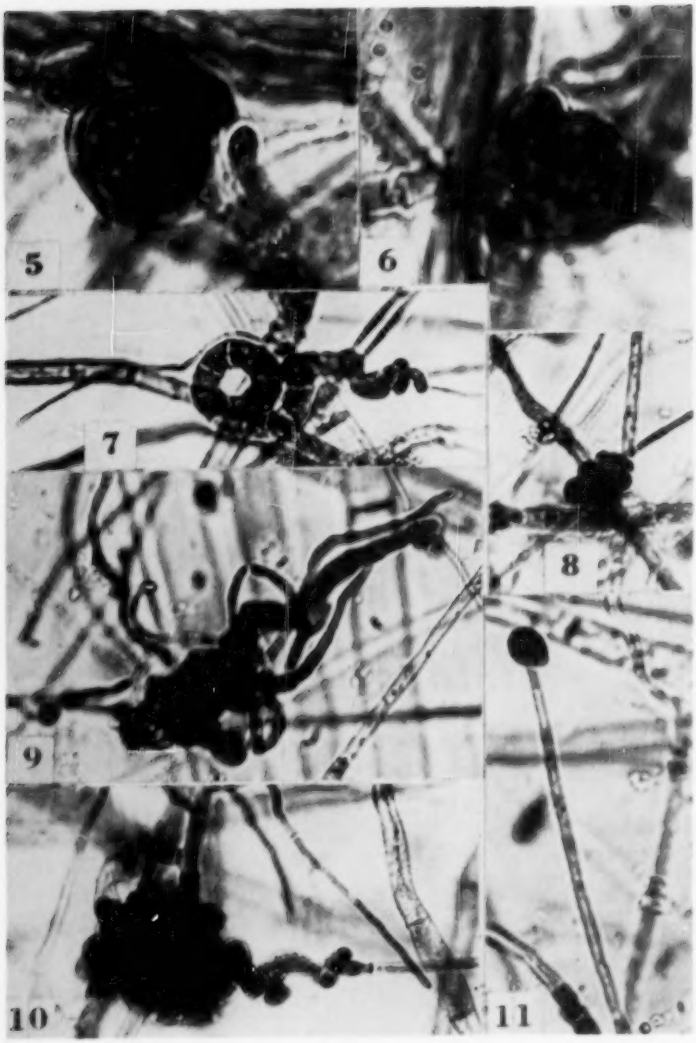
At a slightly later phase, the archicarp becomes differentiated into a terminal trichogyne consisting of from 5 to 20 or more cells, a central ascogonial region of from 2 to 4 cells, and a stalk portion of 2 or 3 cells. The cells of the trichogyne lying closest to the ascogonial region are wider than they are long (FIG. 3). Functional trichogynes taper gradually toward the tip. That segment of the trichogyne in contact with the male element has a tendency to form a tight coil around the latter (FIGS. 7, 8, 9, 10). During the early stages of differentiation of the archicarp, the ascogonial cells are characterized principally by their larger size and their tendency to be constricted at the cross septa (FIG. 3). The stalk cells are difficult to characterize at this stage. There is only a gradual diminution in size backward from the largest cell of the ascogonium to the basal stalk cell.

Using the Riddell slide culture technique, it was observed that the archicarps growing on glass surfaces showed certain abnormalities at the trichogyne end. The most commonly observed abnormality was a branching trichogyne. The type of branching observed was similar to that seen in the vegetative hyphae. A bud forms on one of the subterminal cells and develops into another growing point (FIGS. 2, 3). A more curious and less frequently observed variation is the coiling back of the tip of the trichogyne so that it resembles another young archicarp (FIG. 4).

The male element is somewhat more difficult to characterize than is the archicarp. It is a relatively undifferentiated hypha, distinguished principally by a smaller diameter than the vegetative hyphae. Vegetative hyphae measure from 4 μ to 16 μ in diameter, while antheridial hyphae never appear wider than 4 μ and are sometimes only 2 μ in width. From the material examined thus far, it appears that only one cell of the antheridial hypha is involved in plasmogamy. This cell undergoes gradual swelling until it becomes almost spherical (FIGS. 30, 31). Sometime during the course of this swelling, the walls between the antheridium and the trichogyne break down and a protoplasmic contact between the two cells is established (FIGS. 5, 6, 30).

The swollen cell appears (FIG. 30) to be terminal. However, at a point designated (a) in FIG. 30 but not visible there, the antheridial

ing trichogynes. 4. Same as above but showing a naked archicarp with a short trichogyne which has coiled back so as to simulate another young archicarp. Photographed through a phase microscope to bring out more details of the coiled trichogyne tip.



FIGS. 5–11.

FIGS. 5–11. Whole mounts prepared from Shoemaker slide cultures and stained with erythrosin. 5. Trichogyne tip coiled around the periphery of a swollen antheridial cell. 6. Same example as shown in 5, but photographed at a higher focal plane. 7. Slightly differentiated archicarp coil with trichogyne coiling around an antheridial hypha. 8. Undifferentiated archicarp coil with an antheridial

hypha continues on and branches at right angles. One of these branches follows the trichogyne (b) and the other passes in the opposite direction (c).

Since a single apothecium containing only one archicarp produces ascospores of both mating types, both types of nuclei must be present in the ascogonium prior to the time that ascogenous hyphae are developed. Though nuclei have not been seen during the period when plasmogamy occurs, it is assumed that the opposite mating type nuclei are brought together by the fusion of the tip of the trichogyne and the antheridial cell. Very fine pores have been seen in the cross septa of the young trichogyne through which the antheridial nuclei probably pass.

Another structure observable through use of the Shoemaker slide culture technique was a thin hypha terminated by a bulbous swelling (FIG. 11). Such structures were found frequently in all cultures and were once thought to be antheridial hyphae. It now seems certain that they are not antheridial, since the bulbous portion is always terminal as opposed to the intercalary position of the antheridium in the antheridial hypha. In addition, swelling of the antheridium occurs after contact with a trichogyne has been established, whereas the terminal bulbs on these other hyphae develop without such a stimulus. The function of these bulbs is unknown.

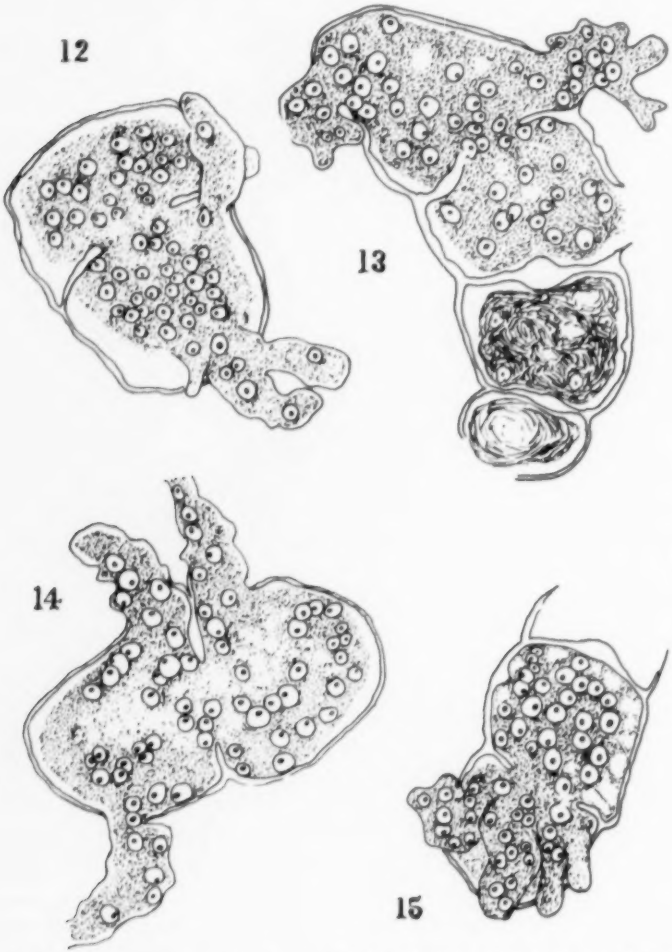
Since the young archicarp is soon covered with ensheathing hyphae, further information on development of the archicarp had to be obtained from sectioned and stained material. Iron hematoxylin was used for differentiating cytoplasm and nuclei, and erythrosin as a counterstain was used to stain the cell walls. Erythrosin proved to be particularly valuable when tracing ascogenous hyphae.

In the youngest sectioned material, the archicarp was multinucleate, and in several early examples the nuclei could be seen to contain up to 3 or 4 darkly staining bodies and no recognizable nucleolus. In these, nuclear division was probably in progress, the dark staining bodies being chromosomes (FIG. 30). With the stained preparations used in this study, no further work on details of nuclear division was possible. However, Olive (1950) has studied nuclear division in the ascus, and he reported that the haploid number of chromosomes in *Anthracobia*

hypha passing through it. 9. Similar to 7, but the archicarp is more advanced, ensheathing hyphae have begun to develop from the basal cells of the archicarp. 10. Naked archicarp with a short trichogyne which has coiled around an antheridial hypha. The antheridial hypha is somewhat swollen at the point where the tip of the trichogyne is in contact with it. 11. Narrow hyphae with bulbous tips. Figs. 5 and 6 photographed and reproduced at × 2000. Figs. 7–11 photographed and reproduced at × 300.

melaloma is 4. This is consistent with the idea that the nuclei observed by this writer in the young ascogonia were actually dividing, since the usual number of chromatic bodies observed was 4.

Sometime during the period prior to the formation of ascogenous hyphae, broad pores develop between the cells in the ascogonial region.



FIGS. 12–15. Camera lucida drawings of sectioned and stained material showing especially the early stages in the formation of the primary ascogenous hyphae. Drawn at × 2000 and reproduced at × 1000.

Protoplasm and nuclei could be seen passing through these pores (FIGS. 12, 13, 14, 15).

When the ascogenous hyphae begin to bud out, the ascogonial cells are filled with large nuclei ranging from 1.5 μ to 3.2 μ in diameter. The peripheral cytoplasm and nuclei migrate out into the developing ascogenous hyphae. The ascogonial cells gradually become devoid of contents, leaving a central core of nuclei and cytoplasm which gradually becomes smaller as more nuclei continue to enter the ascogenous hyphae (FIGS. 15, 20, 23).

Just before the archicarp is finally crushed by the surrounding hyphae, the ascogonial cells are devoid of contents with the exception of a few small nuclei (FIG. 24).

In all sectioned material, the cross walls of 5 to 8 of the more proximal cells of the trichogyne appear swollen toward their center. In side view, the swellings appear as large hemispherical pads whose flat surfaces are adajacent to the opposite faces of the cross walls. These persist throughout the time that the archicarp is visible (FIGS. 13, 16). Close examination of these pads revealed no fine pores connecting the cells of the trichogyne. If it is assumed that plasmogamy is carried out by the mechanism described above, the nuclei must migrate into the ascogonium before the pads form.

Another characteristic which differentiates the trichogyne from the ascogonium is the early breakdown of the contents of the former. The protoplasm loses its granular appearance, and the nuclei disappear (FIGS. 13, 16).

Up to the time that ascogenous hyphae are developed, it is difficult to differentiate the stalk cells from the ascogonial cells. Stalk cells retain a granular protoplasm and contain nuclei which are as large as those in the ascogonial cells. One distinguishing characteristic of the stalk cells is the presence of broad hyphal branches projecting out from them. These hyphal branches are also filled with large nuclei. Such a branch may be distinguished from an ascogenous hypha by the presence of a septum at the point where the branch arises from the stalk cell of the archicarp. A septum is never formed at this point in an ascogenous hypha. However, a stalk cell giving rise to one of these branches may be connected to the adjacent ascogonial cell by a wide pore in the cross septum (FIG. 29). This indicates that no clear distinction can be made between the cells of the ascogonium and those of the stalk. The one distinguishing characteristic of the ascogonium is the ability of its cells to bud out ascogenous hyphae. However, the above observations suggest that the nuclei in the stalk cell adjacent to

the lowest ascogonial cell may pass into the ascogenous hyphae and take part in the formation of asci.

The ascogenous hyphae first appear as large, thin-walled protrusions at various points on the walls of the ascogonial cells. They frequently arise at points adjacent to the cross walls of the ascogonial cells (FIGS. 12, 13, 14). Gwynne-Vaughan (**1937b**) reported that 1, 2, or possibly more cells in the ascogonial region may be involved in the formation of ascogenous hyphae. In the material observed here, 2 cells were sometimes involved, but more commonly 3 or 4 cells gave rise to ascogenous hyphae.

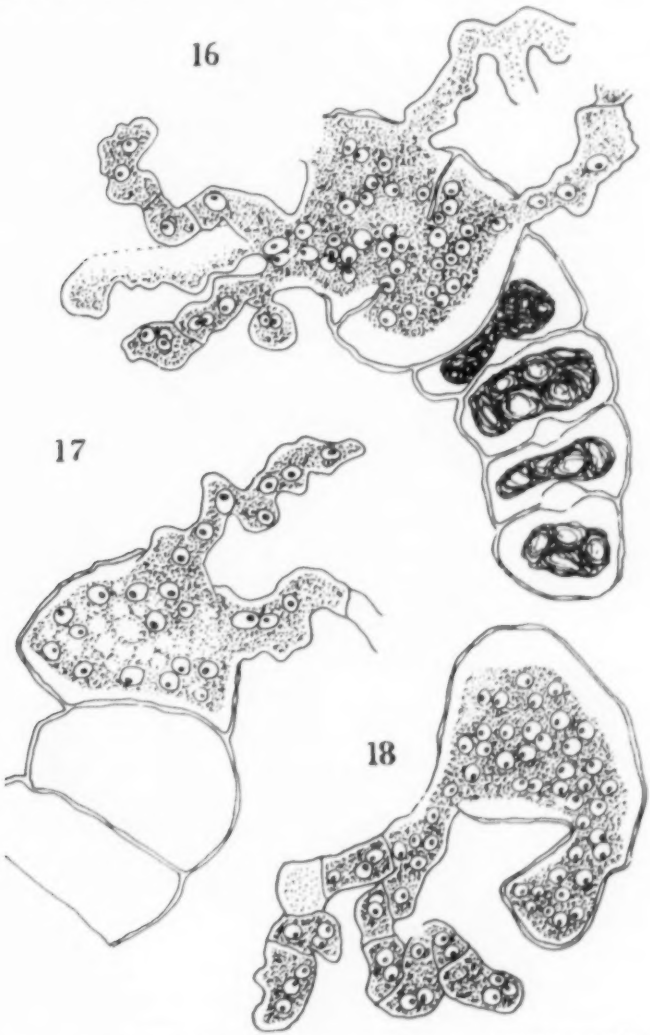
The primary ascogenous hyphae may emerge as simple protrusions (FIG. 12), or they may branch profusely so as to take on an amoeboid character (FIGS. 13, 15). Wilson (**1952**) has observed a stage in the development of the ascogenous hyphae of *Pyronema confluens* in which the first buds had formed but were as yet devoid of nuclei. Such a stage has not been observed in the ascogenous hyphae of *Anthracobia melaloma*. Migration of nuclei and protoplasm occurs immediately. Sometimes the nuclei move into the hyphae in pairs, but this pairing is not a constant feature and perhaps has no functional or pattern significance.

Wilson (**1952**) has shown that following migration of the nuclei into the ascogenous hyphae of *Pyronema confluens*, a simultaneous division of the nuclei is followed by the formation of a septum between the daughter nuclei. Each of the resulting cells would, therefore, contain 2 nuclei, with the exception of the distal and proximal cells. Though nuclear division has not been observed in the ascogenous hyphae by this writer, no such process as described for *P. confluens* is believed to occur in *Anthracobia melaloma*.

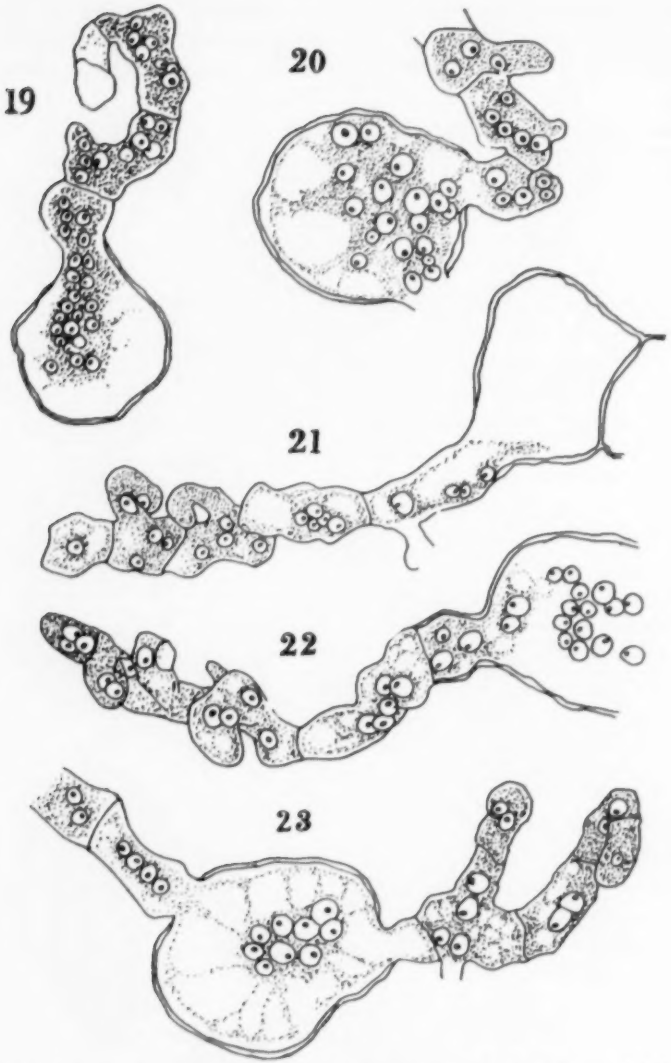
The ascogenous hyphae elongate and undergo septation rapidly, and it is difficult to obtain intermediate stages between the initial protrusions and the elongated and septate hyphae. The few intermediate examples that were seen (FIGS. 16, 17, 18) would indicate that septation started at the distal ends of the hyphae and progressed backwards. In addition, septation apparently occurs at random with reference to the number of nuclei enclosed. The number of nuclei within each resultant cell varies from 2 to 7, and in older hyphae single nuclei or small groups of nuclei were often observed which did not seem to be taking part in the formation of asci (FIG. 24).

The beginnings of conjugate nuclear division and the formation of croziers are the next major changes which occur in the ascogenous hyphae. Sometimes this occurs when a hypha is relatively short. In

such cases, the croziers develop from the terminal cells first (FIGS. 23, 25). More commonly, the primary ascogenous hyphae grow out to the margin of the apothecium before the nuclei begin to divide conjugately.



FIGS. 16–18. Camera lucida drawings of sectioned and stained material showing especially stages in septation of the primary ascogenous hyphae. Drawn at × 2000 and reproduced at × 1000.



FIGS. 19–23. Camera lucida drawings of sectioned and stained material showing early stages during the formation of the secondary ascogenous hyphae. Drawn at × 2000 and reproduced at × 1000. 19–22. Successive stages in the formation of laterals or secondary ascogenous hyphae. 23. Crozier formation at the terminal points on the primary ascogenous hyphae.

On these longer hyphae, lateral outgrowths develop. In the early stages of development, the laterals or secondary ascogenous hyphae show little tendency to bend over (FIGS. 19, 20). In more advanced stages, the laterals have a definite tendency to hook over (FIGS. 21, 22).

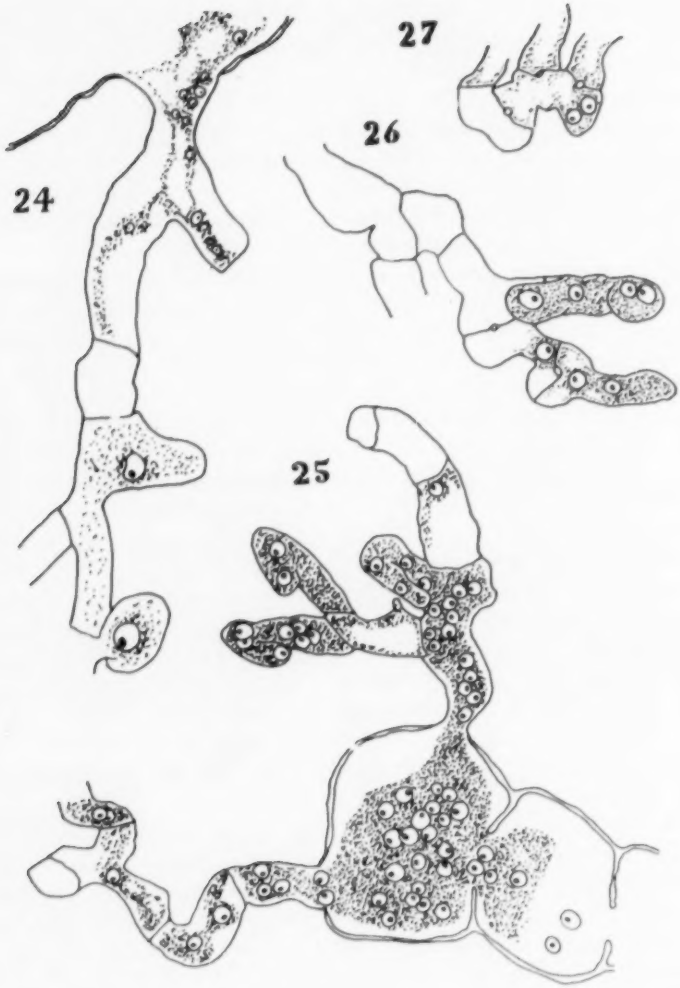
A single cell which may contain from 2 to 7 nuclei has never been seen to put out more than one branch bearing conjugately dividing nuclei. Although no nuclear divisions were seen in the ascogenous hyphae, conjugate division of nuclei is assumed to occur on the basis of observations on development and proliferation of croziers.

The primary ascogenous hyphae rapidly become devoid of contents, and in older sections these structures are no longer visible. Only the apical portions of the secondary ascogenous hyphal system remain active. Growth is indeterminate in these younger portions and proceeds by proliferation of the croziers. In the simplest cases of this type, the nuclei divide in the crozier to form the characteristic binucleate penultimate cell. However, the nuclei in this cell do not fuse and the penultimate cell elongates only about 20 μ, and then forms another crozier. Meanwhile the terminal cell and the adjacent antepenultimate cell fuse. The penultimate cell may proliferate many times before nuclear fusion and the development of an ascus occur.

Branching of an ascogenous hypha containing conjugately dividing nuclei most commonly occurs by the sprouting of the antepunultimate cell (FIG. 26). This cell presumably has the full nuclear complement since the ultimate cell fuses with it. The nuclei in this branch may then continue to divide conjugately forming another series of proliferating croziers. Another type of branching occurs in which the antepunultimate cell continues apical growth and the penultimate cell takes the position of the branch (FIG. 27).

To determine the number of times an ascogenous hypha branches, the number of asci in a single apothecium was estimated. In approximately an eighth of an apothecium which was 0.7 mm in diameter, 250 asci were counted. This would give an estimate of 2000 asci in a single apothecium. Considering that there are an average of 3 ascogonial cells and 3 ascogenous hyphae arising from a single ascogonial cell, a single ascogenous hypha would have to branch over 200 times to yield this many asci. However, this is an estimate from an apothecium which has as yet undergone no marginal growth. Extrapolating from the figures obtained above, each ascogenous hypha would have to branch over 4000 times in an apothecium 3 mm in diameter.

THE STERILE PORTION OF THE APOTHECIUM: Before plasmogamy occurs, branches develop from the stalk cells of the archicarp. These



FIGS. 24–27. Camera lucida drawings of sectioned and stained material showing later stages during formation of the secondary ascogenous hyphae. Drawn at × 2000 and reproduced at × 1000. 24. An older primary ascogenous hypha now almost devoid of contents. 25. Terminal and lateral crozier formation observed in the same ascogenous hypha. 26. A terminal section of a secondary ascogenous hypha showing terminal proliferation and branching. 27. A small section of a secondary ascogenous hypha which is branching repeatedly.

branches grow rapidly, ramify repeatedly, intertwine, and finally form a closed sheath around the archicarp. The sheath hyphae remain appressed to the archicarp as they grow. Since the sheath hyphae grow very rapidly, it is difficult to demonstrate their attraction toward the archicarp except in the region of the trichogyne. In the more distal regions of the trichogyne, the sheath hyphae never become very densely interwoven and the hyphae can be more easily traced (FIG. 1).

After the archicarp had become enclosed, Gwynne-Vaughan (**1937b**) noted cessation in growth of certain of the sheath hyphae in what was to become the upper portion of the apothecium. This cessation of growth was noted in a particular zone. The reader is referred to Gwynne-Vaughan's figures 11 and 12 for illustrations of this point. These terminal hyphae are the first paraphyses and hairs. The hair cells are broader (4–5 μ in diameter) than the paraphyses (1.3–1.5 μ in diameter), and the former have much thicker, and more pigmented cell walls. In addition, the hair cells are soon devoid of protoplasm, as contrasted with the richly protoplasmic paraphyses.

Before the hymenium becomes exposed, the paraphyses are arranged as numerous layers of radially arranged hyphae. Each layer converges at approximately 45° on a common point which is the central axis of the apothecium. The apices of the excipular cells which surround the region where the paraphyses are developing differentiate into hairs. These hairs converge over the paraphyses and meet at a common point at the top of the apothecium. The excipular cells make up the outer and lower portions of the apothecium (FIG. 35). Even at this early stage, the cells of the ectal excipulum have lost the appearance of having been a filamentous system and they appear vacuolate. Following Korf's (**1951**) adaptation of Starbäck's (**1895**) key to tissue types, the ectal excipular tissue of *Anthracobia melaloma* in the region where the hairs are developing would be classified as textura prismatica. In the ectal excipular region below the point where the hairs are arising, the tissue would be classified as textura globosa. The majority of the medullary excipulum would be classified as textura intricata. What protoplasm remains in the excipular cells is concentrated in the peripheral regions (FIGS. 32, 33). These cells are about the same diameter as the hairs or are slightly broader (4.0–5.0 μ in diameter). Certain of the lower excipular cells may continue to grow and invade the substratum. Corner (**1929**) has suggested that such hyphae function to give added support and nourishment to the developing apothecium.

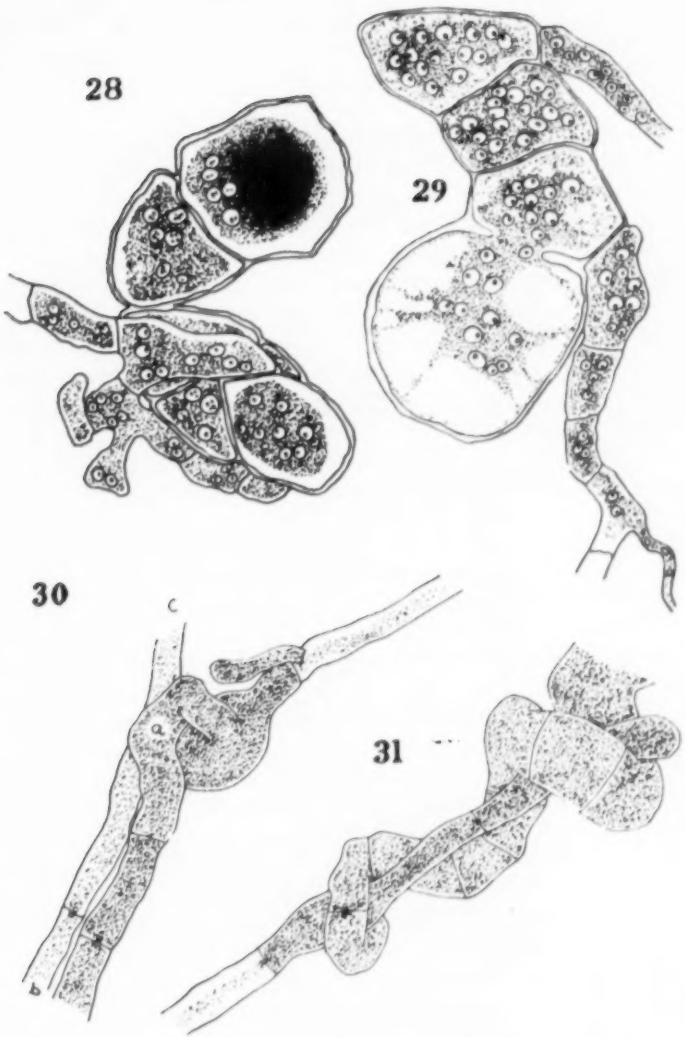
This same writer (**1929**) has divided the Discomycetes into 3 groups on the mode of development of the apothecium. He has classified

Anthracobia melaloma as showing the "hemiangiocarpic" type of development. In forms of this type, the hyphae which arch over the archicarp are of limited growth from the very beginning and do not form a closed sheath. This writer has observed that before any differentiation occurs, the sterile hyphae form a closed sheath. The elements which do not merge are the first formed paraphyses and hairs. *Anthracobia melaloma* shows a condition which is probably intermediate between Corner's "angiocarpic" and "hemiangiocarpic" development.

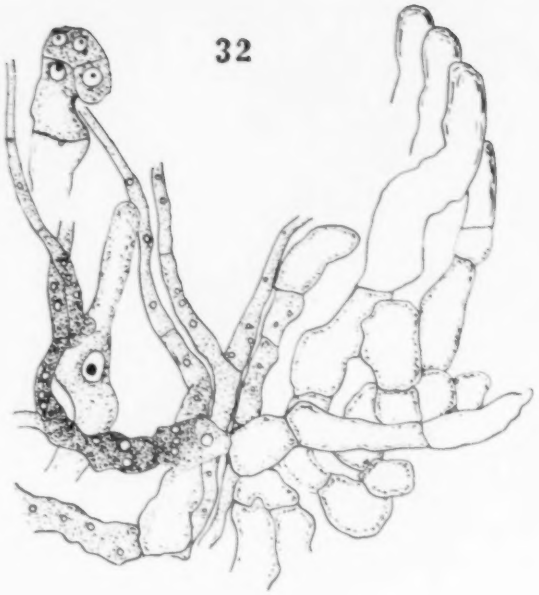
Gwynne-Vaughan (**1937b**) noted the presence of broad hyphae branching from some of the lower cells of the archicarp. She apparently observed these only after the time that ascogenous hyphae had begun to develop. In her Fig. 12 she shows one of these broad hyphae arising from a stalk cell of the archicarp. This stalk cell is apparently not connected to either of the adjacent cells by a broad pore. She suggested that such broad hyphae contribute to the formation of additional paraphyses. Rather than being formed subsequent to the formation of the sheath, these are probably the first branches which developed from the stalk cells of the archicarp. These stalk branches have been noted in all sectioned material here (FIGS. 28, 29), and they are the only hyphae, aside from the asogenous hyphae, which have been seen to branch from the archicarp. Moreover, they are always found projecting from the lowest cells of the archicarp, and these latter are the only cells observed in whole mounts which give rise to ensheathing hyphae. At their bases, these stalk branches are approximately 4 times as broad as the surrounding sheath hyphae to which they probably give rise. In addition, the basal cells of these branches contain nuclei which are as large as those nuclei in the ascogonium. By analogy it seems that the stalk cells and these broad hyphae are the focal points from which the sterile portion of the apothecium is developed, just as the ascogonium is the focus for the ascogenous system.

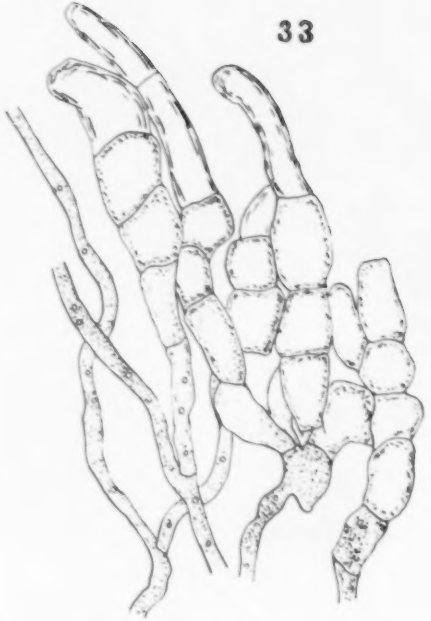
After the first paraphyses have formed and the ascogenous hyphae have begun to push through them, the archicarp degenerates, and additional paraphyses and excipular cells are elaborated by portions of the sheath hyphae in a thin zone between the paraphyses and the excipulum (the medulla). These hyphae appear darkly stained and the nuclei are prominent, though they measure only about 1.0 μ in diameter. These darkly stained hyphae can easily be traced to paraphyses, exciple, and hairs (FIG. 32). As more paraphyses are added and as the ascogenous hyphae develop, the hairs converging over the apothecium are pushed upward and outward, exposing what now appears to be a flat hymenium.

Marginal growth of the apothecium is essentially a sympodium



FIGS. 28–31. 28, 29. Camera lucida drawings of sectioned and stained material showing ascogonial and stalk cells with the first-formed stalk branches. Drawn at × 2000 and reproduced at × 1000. 30, 31. Camera lucida drawings of the same specimens shown in Figs. 5, 6, and 10 resp. Drawn at × 2000 and reproduced at × 1000.





FIGS. 32–33. Camera lucida drawings of sections through the medullary region of the apothecium. Drawn at × 2000 and reproduced at × 1000. 32. Shows especially dark staining hyphae which give rise to additional paraphyses and excipular cells. 33. Section through the ascocarp at the margin, showing sympodial branching of the medullary hyphae.

(FIG. 33). A medullary hypha has become differentiated at its apex into excipular cells and ultimately into a hair. Branches behind the point of differentiation probably maintain the terminal growth of the hypha.

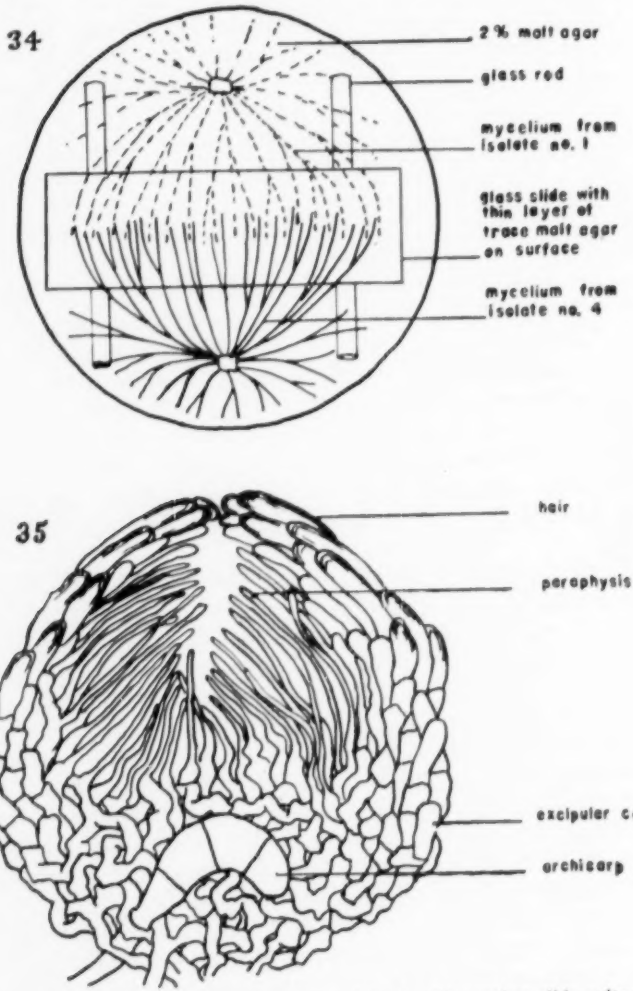
HETEROTHALLISM AND ITS RELATION TO ASCOCARP FORMATION: Gwynne-Vaughan (**1937b**) reported that when mycelia from single spores were paired, ascocarps were obtained in about 50% of the cultures. She concluded from this observation that the fungus was heterothallic.

Single ascospore cultures were obtained from apothecia which had developed from a cross between isolates 1 and 4. These later cultures also fell into 2 groups as regards mating type, and when they were backcrossed to the parent isolates (1 and 4), they invariably produced apothecia with either one mating type or the other. Bipolar heterothallism appears to be represented here.

In June, 1954, another collection of *Anthracobia melaloma* was made by Mr. W. C. Denison of this department during a collecting trip in the Adirondack State Park. This collection afforded an opportunity to determine whether genes determining mating type were members of a multiple allelic series. Single spore cultures were made in the usual way and these were crossed with isolates 1 and 4. In all cases these later cultures fell into one or other of the 2 original classes. The results are consistent with the interpretation that *A. melaloma* contains two mating types that are genetically differentiated by a single allelic pair of genes.

Gwynne-Vaughan and Williamson (**1930**) reported that in *Humaria granulata* archicarps developed in single spore cultures, but they produced apothecia only after anastamoses had occurred between mycelia of the two mating types. Archicarps were produced by both mating types. Their conclusion that plasmogamy occurred by hyphal anastamoses was based on (1) the absence of an antheridium, (2) the occurrence of hyphal anastamoses in mixed cultures, and (3) the absence of a trichogyne. Gwynne-Vaughan (**1937b**) reported that in *Anthracobia melaloma* fusion may occur between a vegetative hypha and the stalk of the coiled archicarp branch, or between vegetative hyphae and branches from the proximal cells of the archicarp. She suggested that it was through such fusions that archicarps received nuclei of opposite mating type. She did not believe that the trichogyne functioned in plasmogamy. Before functional trichogynes were seen by this writer, it was thought that plasmogamy might occur either by anastamoses between hyphae of opposite mating type as described for *Humaria granulata* or as described by Gwynne-Vaughan for *Anthracobia melaloma*.

Since hyphal anastamoses occur readily between hyphae of the same mating type and since the 2 types of mycelia cannot be differentiated morphologically, it is difficult to prove that anastamoses do occur between hyphae of opposite mating types. The modified Riddell slide



FIGS. 34–35. 34. Top view (diagrammatic) of a Shoemaker slide culture. 35. Schematic representation of a longitudinal section of a young ascocarp before the hymenium becomes exposed.

culture technique was used so that anastamosed hyphal strands might be traced to their points of origin. In slide cultures where both blocks were inoculated with the same mating type, many anastamoses developed in the region where intermingling had occurred. Two anastamosed hyphal strands could be traced to either the same or to different points of inoculation. In slide cultures where one block was inoculated with one mating type and where the other block was inoculated with a compatible mating type, fewer anastamoses were seen in the region where intermingling of the two mycelia occurred. Two such anastamosed hyphae were always traced to the same point of inoculation.

Because it is difficult to trace the origin of two anastamosed hyphae and because hyphal fusions are frequently observed in cultures containing only one mating type, hyphal tips were isolated from cultures containing both mating types. If any of the resulting hyphal tip cultures were to produce apothecia, one might plausibly infer hyphal anastamoses between the 2 mating types.

In the first experiment of this type, the 2 mating types were inoculated at adjacent points near the center of a petri plate so that when growth started, intermingling would occur immediately. Hyphal tips were taken at 3 different times after inoculation. The first group of 10 tips was taken 2 days after inoculation. At this time the hyphae had developed to about half of the distance between the point of inoculation and the edge of the plate. Ten tips were taken a day later. The hyphae had, by this time, reached the edge of the plate. Finally, after 6 days (the time usually required for the appearance of archicarps) a circular disc of agar and mycelium was cut out of the petri dish and was placed in a larger petri dish containing a thin layer of malt agar. About 8 hours after this second inoculation, sufficient marginal growth had occurred to permit the removal of hyphal tips. The above procedure improved the chances of picking up the heterocaryon in case marginal growth was faster than nuclear migration.

Of the 30 cultures made from hyphal tips, none produced apothecia. However, the original mixed culture produced numerous apothecia.

In a second experiment, the source of inoculum was an agar block on which was growing an older mixed colony producing mature apothecia. This block of inoculum was placed in the center of a petri dish containing malt agar. Thirty hyphal tips were made from the hyphae growing out from this block. Again none of the resulting cultures produced apothecia. Moreover, when 20 of these hyphal tip cultures were combined with cultures 1 or 4, apothecia were produced with either one mating type or the other.

Failure to show hyphal anastamoses between opposite mating types and the discovery of functional sexual structures led to an effort to determine whether archicarps were produced by only one or by both mating types. Shoemaker slide cultures were again used. In addition to growing a single spore culture alone, inocula from cultures made from different ascospores, but of the same mating type, were placed in the same petri dish. After 6 days, plates containing a culture of isolate 1 or 1 plus another culture of the same mating type, produced archicarps. Plates containing a culture from isolate 4 or 4 plus another culture of the same mating type did not produce archicarps. In later experiments of this type slides bearing isolate 4 were fixed and stained after intervals longer than 6 days. Slide cultures of isolate 4 which

TABLE I

TIME AND TEMPERATURE AS THEY INFLUENCE ASCOCARP AND ASCOSPORE PRODUCTION IN ANTHRACOBIA MELALOMA GROWN ON 2% MALT AGAR IN PETRI DISHES IN THE DARK

Temperature (C)	No. of days in incubator before appearance of apothecia	No. of days in incubator before appearance of mature ascospores*	
12°	15	—	not seen after 25 days
15°	11	—	
18°	11	—	
21°	9	22	
24°	9	18	
27°	9	18	
30°	9	18	

* Check for ascospores made by removing an apothecium from a petri dish culture and mounting on a slide for observation under the microscope.

were 8 or 9 days old produced archicarps. The number of archicarps seen in cultures containing a single mating type is considerably smaller than that seen in a mixed culture.

No reference is made to antheridia or antheridial hyphae in these experiments since they are relatively undifferentiated and cannot be detected except when seen in contact with a trichogyne. Since archicarps are produced by both mating types, it is probable that antheridial hyphae are also produced by both mating types.

THE INFLUENCE OF LIGHT AND TEMPERATURE ON FRUITING: Robinson (**1926**) pointed out that light was necessary for the formation of archicarps and apothecia in *Pyronema confluens*. Petri dish cultures of *Anthracobia melaloma* were incubated at 24° C for 9 days. Half of the plates were wrapped in cellophane and half were wrapped in heavy black construction paper. A 40 watt light bulb was installed in the incubator as a light source. Mature apothecia were formed in both

sets of plates. There were no observable differences between the apothecia produced in the two sets of plates. Light does not appear to influence fruiting.

In another experiment, petri dish cultures were incubated at temperatures ranging from 12° C to 30° C. TABLE I indicates the response to temperature.

These data would indicate that any temperature ranging from 24° C to 30° C is favorable for development of apothecia and ascospores. The number of apothecia produced is a better index at higher temperatures. Numerous apothecia were produced at 24° C whereas the number was considerably fewer in plates grown at higher temperatures. In plates kept below 24° C the number of apothecia was again considerably fewer. 24° C seems to be the optimum temperature within the range tested both for rapidity of development and for the number of apothecia produced.

DISCUSSION

One point which should impress the reader is the structural variability of the archicarp of *Anthracobia melaloma*. Besides the variation shown by those archicarps growing on glass, a wide range in the size and number of cells making up a normal archicarp was observed. Where plasmogamy has been observed, the trichogyne varies considerably in length, depending probably upon the lapse of time between archicarp formation and contact of the trichogyne with an antheridial hypha. This variability may explain why fertilization has been overlooked in this species for so long. Often the entire coil is tightly wound around an antheridial hypha, and the tip of the coil is obscured (FIG. 8). Most successful archicarps presumably come in contact with an antheridial hypha early in their development, and in these cases the fusion is soon obscured by the formation of ensheathing hyphae. Since the antheridial hypha is a relatively undifferentiated structure, it would certainly be overlooked as a sex organ unless a trichogyne were seen in contact with it.

The type of fertilization observed in *Anthracobia melaloma* is unique among Operculate Discomycetes. In all other cases where functional sex organs have been demonstrated, the antheridium is recognizable as a differentiated structure. Swelling occurs in the antheridial cell of *A. melaloma* only after contact with the trichogyne has been made.

Aberrations at the trichogyne end of the archicarp of *A. melaloma* are of particular interest since a functional trichogyne is characteristic of the species. Gwynne-Vaughan (**1913**) showed that the trichogyne of *Lachnea cretea* developed branches. However, she reported no

antheridium, and the trichogyne was never observed to fuse with another hyphal element. It is not clear whether she saw branching trichogynes in mature apothecia or not. In the light of these more recent observations on *Anthracobia melaloma*, the possibility that a plasmogamy between the trichogyne and the antheridium does occur in *Lachnea cretea* should not be excluded.

Ternetz (**1900**) reported that the tip of the trichogyne of *Ascophanus carneus* sometimes bent over to form a structure resembling another archicarp. However, Cutting (**1909**) failed to find secondary archicarps, and Ramlow (**1914**) suggested that Ternetz' material was aberrant. Observations on *Anthracobia melaloma* would indicate that Ramlow's contention was probably correct. The secondary coils produced by archicarps of *A. melaloma* growing on glass are unlike the coiled tip of a trichogyne which is wrapped around an antheridial hypha. The trichogyne cells in the "secondary coil" (FIG. 4) have a greater diameter and the coiling is more irregular.

The origin of the broad pores connecting the ascogonial cells or of the pads between the cells of the trichogyne is not fully understood. Fitzpatrick (**1918**) suggested that the swellings or "pads" in the cross septa of the archicarp of *Rhizina undulata* were a stage in the gelatinization of the wall material. He noted that these swellings disappeared a little later, leaving wide pores between the cells. In *Anthracobia melaloma* it is difficult to tell whether pads were once present between the cross walls of the ascogonial cells. In all sectioned material, the broad pores were already present. In whole mounts, pads have been seen in most of the proximal cells of the archicarp. By deduction from Fitzpatrick's observations and those made here, pads are probably formed between the cells of the ascogonium sometime during the early development of the ascocarp but after plasmogamy has occurred.

Ramlow (**1914**) working on *Ascophanus carneus* and Schweizer (**1923**, **1932**) working on *Ascobolus citrinus* and *Ascobolus furfuraceus* suggested that only one of the cells connected by the broad pores should be considered an ascogonium. The other cells were thought to be antheridial. In all of the papers cited above, the authors believed that the trichogyne was non-functional. However, plasmogamy as observed in *Anthracobia melaloma* would make Ramlow's and Schweizer's suggestion untenable. The pores permit free movement of nuclei from one cell of the ascogonium to another and pads block movement of nuclei or cytoplasm from the trichogyne into the ascogonium. How such modifications in the cell walls contribute to the development of the ascocarp is not known.

The only paper that discusses the ascogenous hyphae of any operculate discomycete in detail is that by Wilson (1952) on *Pyronema confluens*. Certain similarities in the development of the ascogenous hyphae of *Pyronema* and *Anthracobia* are worth mentioning. It is fairly easy to distinguish a primary and a secondary ascogenous hyphal system in both cases. The primary system consists of the elongations of the first buds which emerge from the ascogonial cells. The secondary system consists of the laterals which emerge from the cells of the primary hypha. In both cases, these laterals contain 2 nuclei which divide conjugately to form a true dicaryotic mycelium. The primary ascogenous hyphae are very extensive in both cases, ramifying through most of the medullary portion of the apothecium. Excluding marginal growth, these primary hyphae distribute the contents of the ascogonium throughout the area which becomes the base of the hymenium. Septation in the primary ascogenous hyphae prior to the formation of secondary branches is another characteristic common to both fungi.

SUMMARY

Anthracobia melaloma is a small operculate discomycete which is found most frequently on burnt wood. It grows and fruits readily in pure culture. The species is heterothallic.

Arichicarps are produced by both mating types when these are grown separately. Antheridia cannot be distinguished except when they are in contact with a trichogyne.

Plasmogamy is effected by the coiling of the tip of the trichogyne around an antheridial hypha. One cell of the antheridial hypha, the one in contact with the very tip of the trichogyne, swells until it is almost spherical. The walls between this swollen cell (the antheridium) and the trichogyne break down permitting the interchange of nuclei and cytoplasm.

The archicarp can be separated into 3 distinct regions just prior to the formation of ascogenous hyphae. The ascogonial cells have very large pores in the cross walls. The cells of the trichogyne can be distinguished by the amorphous structure of the protoplasm and the swellings in the cross walls of the 6 to 8 cells nearest to the ascogonium. The stalk cells are distinguished best by the broad hyphae branching from them.

Ascogenous hyphae develop from 2, 3, or 4 ascogonial cells. These rapidly spread throughout the young apothecium and become septate. Branches arise from the cells of these primary ascogenous hyphae and 2 nuclei migrate into each of these branches. The lateral branches

immediately bend over forming characteristic croziers, and conjugate division of the 2 nuclei in these laterals presumably occurs. Asci do not form immediately after the first croziers are developed. Instead, the nuclei in the penultimate cell usually divide conjugately again. This process of conjugate division in the penultimate cell may occur many times before an ascus is finally formed.

The hyphae giving rise to the sterile portion of the apothecium develop from the stalk cells of the archicarp. These are broad hyphae which contain a darkly stained protoplasm and nuclei of the same size as the nuclei in the ascogonial and stalk cells.

The formation of the cortex and paraphyses is the result of sympodial growth of the sheath hyphae as described by Corner (**1929**). The hairs are merely terminal cortical cells which differentiate further by elongation and by darkening and further thickening of the walls.

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DEVELOPMENT OF SYNCHYTRIUM INNOMINATUM IN RELATION TO OTHER SIMILAR SPECIES [1]

JOHN S. KARLING

(WITH 31 FIGURES)

Synchytrium innominatum was first described by Farlow (**1885**) on *Malacothrix* sp. from California and later reported by McMurphy (**1913**) on *Agoseris grandiflora* near Stanford University. These are the only published reports of its hosts and occurrence, but in examining collections of *Synchytrium* in the herbarium of the University of California at Berkeley the author found specimens labelled *S. innominatum* on *Boisduvalia densiflora, Microseris biglowii, M. aphantocarpa* and *Scorzonella laciniata,* which so far have not been recorded as hosts of this fungus. These collections were studied to determine whether or not they are identical with *S. innominatum,* if more than one species is present on these hosts, and if a prosorus occurs in their summer cycles. However, it was soon found that to answer these questions and to compare the species adequately with *S. innominatum,* it was first necessary to study the latter species on one of its known hosts, because *S. innominatum* is incompletely known with respect to its life cycle, variations, gall formation, and cellular effects on the host. While such a study was essential to a careful comparison, it was needed also to amplify our knowledge of *S. innominatum.* At the same time it provided the opportunity for determining the origin of the deep-seated or largely-embedded type of gall which is induced by several species of *Synchytrium.* The origin and development of protruding and largely-superficial galls, as induced by *S. australe,* for example, have been determined by the author (**1954**), but little is known about the genesis of deep-seated galls such as those caused by *S. innominatum, S. taraxaci,* and other species. Accordingly, fixed and stained sections of *S. innominatum* on *Agoseris grandiflora* were obtained for study of these problems. The present

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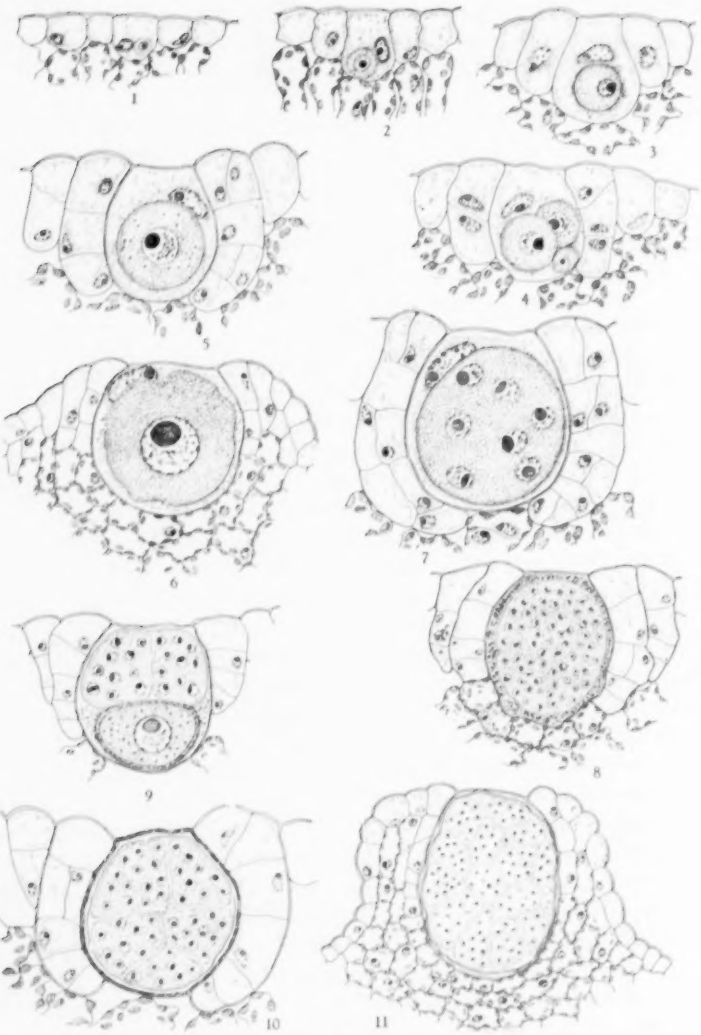
contribution will relate first to gall formation, then to the life cycle and development of *S. innominatum*, and finally to a comparison of this species with those on the other hosts reported above.

HOST REACTION AND DEVELOPMENT OF S. INNOMINATUM

Synchytrium innominatum occurs on both surfaces of the lower leaves and on the petioles and stems of *A. grandiflora*, and causes marked discoloration, stunting and crinkling when very young leaves are abundantly infected. Apparently, only epidermal cells are infected because no parasites have been observed in the mesophyll and palisade cells. The earliest stage of infection found so far is shown in FIG. 1, in which the minute parasite lies beside the host nucleus in the base of a lower epidermal cell. Even at this early stage the host cell has enlarged slightly inward and is distinguishable from the adjacent epidermal cells. This is the first visible reaction of the host to infection. A later stage is shown in FIG. 2 in which the infected upper epidermal cell has increased in diameter and enlarged farther inward on the palisade cells and is beginning to expand at its base. The adjacent epidermal cells also have elongated inward, and it is to be noted that neither the infected nor adjacent cells bulge outward. This early inward growth foreshadows the development of deeply embedded galls. FIG. 3 shows a later stage in which this enlargement is more advanced, and the infected cell has become sac-like and projects inward among the loose mesophyll cells. Multiple infection is quite common in this species (FIG. 4), and as many as 8 parasites of different sizes may occur in a single cell. It is to be particularly noted in this figure that the adjacent elongate epidermal cells have divided almost transversely, and in 4 of the cells the daughter nuclei appear to be in the telophasic reconstruction stage. The elongation inward and division of such cells around the host cell initiates the development of the gall sheath, which may become one to several cells in thickness by the time the gall is mature. Frequently, farther removed epidermal cells may elongate also, which shows that the stimulus of infection may extend beyond the immediately adjacent cells. This is apparent in FIG. 4 and particularly pronounced in FIGS. 5 and 8, in which such cells have divided transversely. The epidermal cells immediately adjacent to the infected one in FIG. 5 have elongated inward along the latter's sides and are beginning to curve around its base. At the same time they have divided transversely and longitudinally. This is even more pronounced in FIG. 7, where derivatives of such cells have enveloped the infected one almost completely except at the base.



FIGS. 1–11.

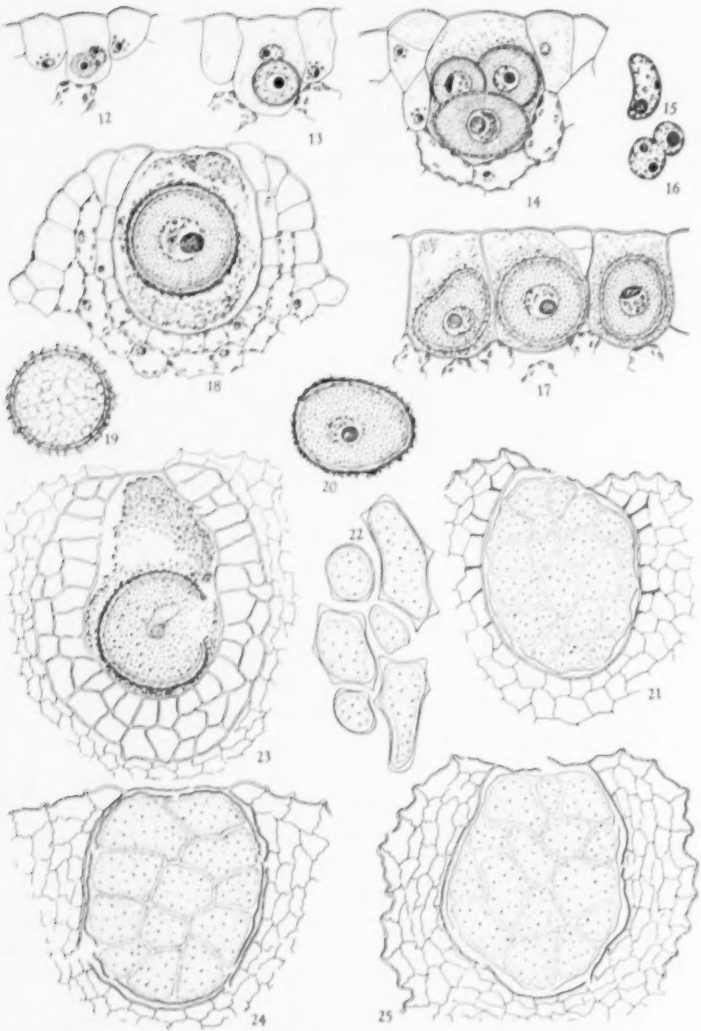
FIGS. 1–11. *Synchytrium innominatum* on *Agoseris grandiflora*. 1. Young parasite in lower epidermal cell, × 269. 2–3. Later stages showing inward growth and expansion of infected cell; adjacent epidermal cells elongating inward also, × 269. 4. Later stage showing multiple infection, further enlargement of infected cell, and division of adjacent epidermal cells, × 269. 5. Later stage showing divi-

At the same time, the palisade and mesophyll cells which impinge on the base of the host cell may become involved in sheath formation also. This is evident in FIG. 7, in which one of the mesophyll cells has divided and the daughter nuclei are in the late telophases of division. In some galls only a few mesophyll or palisade cells seemed to be involved, but in others division of such cells is fairly extensive, as shown in FIGS. 6 and 11. At least the basal and some of the lateral sheath cells contain numerous chloroplasts, which suggests that they have been derived from the palisade or mesophyll layers. On the other hand, it is not improbable that some of the meristematic derivatives of the ingrowing epidermal cells developed plastids. This is suggested by the basal sheath cells with plastids in FIG. 8.

The majority of galls caused by *S. innominatum* on *A. grandiflora* are embedded largely in the host tissue and usually protrude only slightly. As described above, such galls develop principally by the ingrowth and enlargement of the infected epidermal cell, elongation inward and division of adjacent epidermal cells, and division of some palisade or mesophyll cells. So far no division of the infected cell has been seen, and it does not appear to be involved in sheath formation. The sheath appears to be derived from the adjacent epidermal, palisade and mesophyll cells. The inward development of these deep-seated galls is quite different from the superficial ones caused by *S. australe*, for instance, where the infected cell grows outward, divides and usually gives rise to the sheath, according to the author (**1954**). The method of gall formation described above for *S. innominatum* may be the same for most species which induce deep-seated composite galls, but each one must be studied individually. The resting-spore galls of *S. innominatum* are predominantly deep-seated also, and their origin was found to be fundamentally the same as that of the sporangial galls and need not be described again.

Not all galls, however, are deep-seated in the host tissue. Some may protrude slightly (FIG. 6), or quite conspicuously, as shown in FIGS. 11 and 16. In the last two figures the sheaths are 3 to 4 cells thick and composed of derivatives of epidermal and mesophyll cells.

sion of adjacent epidermal cells, × 269. 6. Slightly protruding gall with sheath of derivative cells from epidermis and mesophyll; incipient sorus with large primary nucleus, × 133. 7. Early multinucleate stage of sorus; incipient sheath consisting of derivatives of elongated epidermal cells; divided mesophyll cell at base, × 269. 8. Multinucleate prosorus enveloped by residue, × 174. 9. Small infected cell with sorus of two sporangia and a resting spore; base of infected cell without sheath, × 269. 10. Small sunken gall with sorus of incipient sporangia, × 269. 11. Large protruding gall with a thick sheath, × 100.



FIGS. 12–25.

FIGS. 12–20. *Synchytrium innominatum* on *Agoseris grandiflora*. 12, 13. Initial stages in resting-spore gall and spore development, × 269. 14. Small composite resting-spore gall with three spores, × 269. 15, 16. Enlarged reniform and constricted host nuclei, × 333. 17. Simple unicellular resting-spore galls, × 269. 18. Large composite, protruding resting-spore gall, × 100. 19. Surface view of

These galls are, nevertheless, broadly crateriform at the apex, like the deep-seated ones with the upper part of the infected cell exposed. Both resting-spore and sporangial galls are predominantly composite and multicellular, and *S. innominatum* may be described as compositely dihomeogallic (Karling, **1955b**) in relation to its host. The embedded composite sporangial galls are 105–120 μ high by 90–180 μ broad, and the large protruding ones may be 168–275 μ high by 120–270 μ broad. The composite resting-spore galls vary from 110–140 μ high by 96–120 μ broad. Occasionally, the sheath may be reduced so that the base of the infected cell is only partly enveloped (FIGS. 9, 10). Also, simple and unicellular sporangial galls are formed in rare cases and consist only of the enlarged host cell. Simple resting-spore galls occur more commonly, especially when numerous adjacent epidermal cells are infected, as shown in FIG. 15. The small simple sporangial and resting-spore galls vary from 74–90 μ high by 80–102μ broad.

As to the development of the parasite, it enlarges more slowly than the host cell at first and fills the latter only partly (FIGS. 3–5), with the host nucleus above. However, as the host cell approaches its definitive size the relative sizes change. The thallus of the parasite grows and enlarges until it fills most of the host cell, as shown in FIG. 6. In this figure the thallus or initial cell is almost mature, with a very large primary nucleus, and is enveloped by a small amount of host protoplasm. The host nucleus (FIGS. 6 and 7) in the apex of the cell is markedly enlarged and somewhat reniform in shape with a conspicuous nucleole and numerous densely stained chromatic granules and strands. It may persist in this until the sorus is mature and undergoes cleavage, after which it begins to degenerate and usually becomes obscured by the expanding sporangia and residue.

Following the stage shown in FIG. 6, the primary nucleus divides, and the incipient sorus becomes multinucleate (FIG. 7). Additional mitoses occur until several hundred nuclei are formed (FIG. 8) in the large sori. The incipient sorus in this figure is enveloped by a fairly dense layer of residue, but in other cases little or no residue is present (FIGS. 10, 11). The completion of nuclear division is followed by progressive cleavage, whereby 2–60 multinucleate segments are delimited

resting spore with reticulate adhering layer of residue, × 150. *20.* Cross section of resting spore, × 150. FIGS. *21–23. Synchytrium* sp. on *Microseris aphantocarpa*. *21.* Deep-seated sporangial gall with sorus of sporangia, × 169. *22.* Variations in size and shape of sporangia, × 520. *23.* Deep-seated resting-spore gall with thickened sheath cell walls, × 169. FIGS. *24, 25. Synchytrium* sp. on *Microseris bigelovii*. Deep-seated and protruding sporangial galls, respectively, × 169.

(FIGS. 9–11). From the evidence at hand, it appears that cleavage ceases with the delimitation of multinucleate segments and does not continue until uninucleate "protospores" are delimited as Harper (**1889**) and the author (**1955a**) reported for *S. aecidioides* (*decipiens*) and *S. oxalydis*, respectively.

The sori of sporangia are predominantly ovoid or ellipsoidal, 96–120 μ × 115–150 μ, or oblong with the long axis oriented outward (FIGS. 3, 11), or subspherical (FIG. 10), 74–120 μ, with a thin hyaline wall. They may fill the host cell almost entirely (FIG. 11) or only partly (FIG. 8) and are occasionally enveloped by a layer of host protoplasmic residue. Occasionally, very small sori only 40–50 μ in diameter occur, and these may bear only 2 or 3 sporangia (FIG. 9). Usually 10 to 60 sporangia are formed in the sori, and these are predominantly polyhedral, 28–62 μ in greatest diameter, but vary markedly in shape and size (FIG. 11).

The early development stages of the resting spores and their galls are essentially similar to those of the sori and sporangial galls, as shown in FIGS. 12 and 13, and need not be described in detail. The young resting spore can be distinguished fairly early from the incipient sori by its thicker wall and the presence of numerous granules in the dense cytoplasm. Multiple infections occur here also, and as many as 3 mature resting spores may be found in a host cell (FIG. 14). As the resting spores mature, the wall thickens, and the cytoplasm becomes more densely alveolar and coarsely granular. At the same time the enveloping host cytoplasm becomes denser, while the host nucleus enlarges markedly and becomes somewhat reniform in shape (FIG. 15). Later, it may become slightly lobed or deeply constricted (FIG. 16) and usually persists in this condition until the spores have matured. The host protoplasm condenses as the spores mature and forms a residual layer around them. It may be relatively sparse or abundant and fill most of the remaining volume of the host cell (FIG. 18). The mature spores are spherical to subspherical, 70–90 μ, or ellipsoidal, 40–80 × 60–100 μ, with a smooth, brown wall, 3–4 μ thick, and coarsely granular content. In cases where the enveloping residue is sparse, it appears somewhat as a reticulum on the surface of the spore, and unless it is carefully examined the outer wall of the spore may seem to be reticulate in structure. FIGS. 19 and 20 show such spores in surface view and in cross section. Germination of the spores has not been observed, and it is not known whether they function as sori, prosori or sporangia in this process. Therefore, *S. innominatum* can be included in *Eusynchytrium* only provisionally at present.

SYNCHYTRIUM SP. ON MICROSERIS APHANTOCARPA

Heavily infected bits of the host of this species as well as of those on *Boisduvalia densiflora*, *Microseris biglowii* and *Scorzonella laciniata* were thoroughly soaked in water, fixed in a vacuum to remove air, embedded, sectioned at 7 μ, and stained. The host tissue in such sections was badly shrunken, but the galls, sori, sporangia and resting spores were well enough preserved for determination of the characteristics of the fungi and the structure of the galls. However, it should be remembered that the dimensions of the fungi given below are from such herbarium material, and it is quite possible that they may differ somewhat in living specimens.

The species on *M. aphantocarpa* was collected by H. L. Mason at Almaden, Calif., 4–17–1932, and determined as *S. innominatum* by Lee Bonar. It occurs on both surfaces of the lower leaves and may cause stunting and curling of very young leaves when they are heavily infected. For the sake of brevity this species is described in a diagnostic manner as follows:

Sori usually solitary, oblong to elongate, 130–140 × 198–240 μ, ovoid, 140–152 × 150–170 μ (FIG. 21), occasionally subspherical or spherical, 90–140 μ, with a fairly thick wall; sporangia 8–35 per sorus, polyhedral, usually large, 54–91 μ in greatest diam. (FIG. 21), exceptionally small ones 30–36 μ, with hyaline walls which are usually thickened at the angles (FIG. 22); zoospores unknown; resting spores solitary, subspherical to spherical, 42–66 μ, or ovoid, 40–66 × 48–84 μ (FIG. 23) with a smooth wall, 4–4.8 μ thick, and coarsely granular content, enveloped usually by a large amount of reddish-brown residue which adheres closely to the spore wall and may fill remainder of host cell (FIG. 23); germination unknown.

Compositely dihomeogallic, galls separate and scattered or crowded and confluent, usually deep-seated or largely embedded in host tissue, occasionally protruding conspicuously, broadly crateriform at apex; sporangial galls (FIG. 21) occasionally ruby-red, 170–192 μ high by 200–220 μ broad, exceptional ones up to 400 μ in diam., sheath 2–4 cells thick, sheath cells usually thin-walled and not greatly enlarged; resting-spore galls (FIG. 23) 96–270 μ high by 102–240 μ broad, sheath 2–5 cells thick, inner sheath cells enlarged and thick-walled (FIG. 23).

The galls in the material at hand are usually crowded and frequently confluent, with the result that infected areas of a leaf may be up to 720 μ thick. In cross sections of leaves, the sporangial and resting-spore galls are sharply distinguishable because the sheath cells adjacent to the infected cell in the latter galls are greatly enlarged with thick

walls which stain dark red with safranin (FIGS. 21, 23). They appear to be lignified, but no scalariform perforations have been observed in them. Occasionally, in sporangial galls the radial walls of the sheath cells adjacent to the upper portion of the host cell may be slightly thickened and deeply stained (FIG. 21), but this thickening is not very extensive. The majority of both types of galls are embedded largely in the host tissue, but sometimes they may be largely superficial and protrude conspicuously on the surface of host. In such variations this species is similar to *S. innominatum* on *Agoseris grandiflora*.

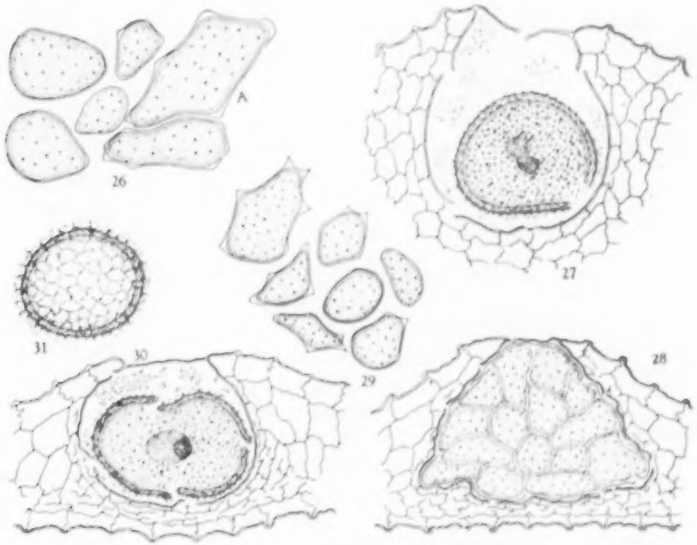
No evidence of a prosorus has been found in this fungus nor in those on *M. biglowii* and *Scorzonella laciniata*, and the initial cells of these species apparently develop directly into sori of sporangia. In this respect, as well as in their inducement of composite sporangial and resting-spore galls on the hosts, they are similar to *S. innominatum* on *A. grandiflora*. In the species on *M. aphantocarpa* the sori and sporangia are usually larger, and its resting spores are generally smaller than those of *S. innominatum*. Nevertheless, they usually fall within the size ranges of those of the latter species. The most conspicuous differences noted so far are the greater size of the galls and the thickened sheath cell walls noted above. However, these are differences in reaction of the host to infection and may not be taxonomically significant so far as the fungus itself is concerned.

SYNCHYTRIUM SP. ON MICROSERIS BIGLOWII

This species was collected by J. P. Tracy, 5–12–1929, in Del Norte Co., and at Trinidad, Humboldt Co., Calif., by H. E. Parks and J. P. Tracy, 5–9–1933. It occurs principally on the lower leaves and petioles and causes discoloration, thickening and curling when very young leaves are heavily infected. The resting-spore galls are frequently crowded and confluent and may be so abundant that they form lavender-violet crusts on the leaves and petioles. In such areas the leaves may be up to 360 μ thick. The characteristics of the fungus are as follows:

Sori subspherical, 98–200 μ, usually ovoid, 144–180 × 175–240 μ (FIG. 24); sporangia 15–48 μ per sorus, very variable in size and shape, polyhedral, usually quite large (FIG. 26A), 54–68 μ in greatest diam., exceptionally large ones, 96–120 μ, small ones, 32–48 μ, with finely granular content and hyaline walls thickened at the angles (FIG. 26A); zoospores unknown; resting spores usually solitary, subspherical, 75–98 μ, usually ovoid, 70–90 × 85–102 μ, with a smooth, reddish-brown wall, 3.8–4.2 μ thick (FIG. 27), enveloping residue sparse or frequently lacking; germination unknown.

Compositely dihomeogallic, galls separate and scattered but usually crowded and frequently confluent; sporangial galls buff to tan in color, usually deep-seated or embedded largely in host tissue (FIG. 24), separate galls on leaves occasionally protruding conspicuously and largely superficial (FIG. 25), 180–228 μ high by 240–408 μ broad, sheath 3–6 cells thick, sheath cells greatly enlarged and thin-walled; resting-spore galls usually deep-seated, occasionally protruding conspicuously, lavender-violet, 100–140 μ high by 160–200 μ broad, sheath 1–4 cells thick, sheath cells enlarged and thin-walled (FIG. 27).



FIGS. 26, 27. *Synchytrium* sp. on *Microseris bigelovii*. 26. Variations in size and shape of sporangia, × 520. 27. Deep-seated resting-spore gall with spore and trace of residue, × 169. FIGS. 28–31. *Synchytrium* sp. on *Scorzonella laciniata*. 28. Sporangial gall with sorus of sporangia, × 250. 29. Variations in size and shape of sporangia, × 520. 30. Resting-spore gall with spore, × 250. 31. Surface view of resting spore, × 250.

As noted above, the sori and sporangia of this fungus may be much larger than those of *S. innominatum* and the species on *Microseris aphantocarpa*. Nevertheless, they vary markedly in size and shape. Also, the isolated galls protrude more conspicuously and may be much larger with a thicker sheath (FIG. 24), and the sheath cells are usually more enlarged than in the previous species. Usually, there is less resi-

due around the resting spores (FIG. 27) or it is frequently lacking. In cases where it is present, the outer wall of the resting spore may appear to be slightly reticulate. On the basis of these differences, particularly the larger sori and sporangia, the fungus may seem to be a distinct and different species, but until such differences have been verified from intensive study of living material, the author does not think it should be regarded as a new species.

SYNCHYTRIUM SP. ON SCORZONELLA LACINIATA

This species was collected by J. P. Tracy, 4–24–1932, near Redway, and by H. E. Parks and J. P. Tracy, 5–16–1942, at Garberville, Humboldt Co., Calif. In the material at hand the galls are abundant on both surfaces of the leaves and on the petioles, but no marked malformation of the infected organs is evident. However, confluent galls often form broad irregular cushion-like enlargements on the leaves and petioles, and in such areas the leaf may be up to 260 μ thick. The characteristics of this fungus are as follows:

Sori usually solitary, subspherical, 65–120 μ, ovoid, 95–114 × 108–150 μ, or broadly pyriform (FIG. 28) with a wall 1.8–2 μ thick; sporangia 3–26 per sorus, highly variable in size and shape, polyhedral, usually 36–56 μ in greatest diam., large ones up to 68 μ, small ones 24 μ, with finely granular content and hyaline walls which are thickened at the angles (FIG. 29); zoospores unknown; resting spores (FIGS. 30, 31) subspherical, 56–95 μ, ovoid, 54–72 × 90–108 μ, with a reddish-brown wall, 3–42 μ thick, enveloping residue sparse or lacking; germination unknown.

Compositely dihomeogallic, galls separate and scattered or crowded and confluent, deep-seated and embedded largely in the host tissue but protruding, usually broadly crateriform at apex; sporangial galls broadly cone- or dome-shaped (FIG. 28), 96–126 μ high by 134–216 μ broad at base with a broad apical pore after dehiscing, sheath 2–4 cells thick, sheath cells usually markedly enlarged and thin-walled; resting-spore galls (FIG. 30) 90–110 μ high by 144–168 μ broad, sheath 1–3 cells thick, sheath cells enlarged and thin-walled.

As described above, the dimensions of the sori, sporangia and resting spores are fairly close to those of *S. innominatum*, but its sori and sporangia are usually smaller than those of the species on *M. aphantocarpa* and *M. biglowii*. The size and shape of the resting spores, however, are similar to those of these two species. The dimensions of its galls also are fairly similar to those of *S. innominatum* but smaller than

those on *M. aphantocarpa* and *M. biglowii*. They differ further in that most of them are not as deeply-seated and project more conspicuously on the surface of the host. The surface of most resting spores of the fungus on *Scorzonella laciniata* appear to be conspicuously reticulate (FIG. 31), and for a time the author thought that this might be a specific spore wall characteristic. However, careful study of a large number of spores has led him to the conclusion that this appearance is due to the tightly adhering but thin layer of residue.

The fungus on *Boisduvalia densiflora* which Lee Bonar determined as *S. innominatum* is a different species, because its initial cell develops into a prosorus. It is similar to the species previously described on the same host by the author (**1956**) who believed it might be identical with *S. epilobii*.

DISCUSSION

It is not certain from this study that the fungi on *Microseris aphantocarpa, M. biglowii* and *Scorzonella laciniata* are identical with *S. innominatum* or new and different species. The size and structure of their zoospores as well as the method of resting-spore germination are unknown. Furthermore, it is not known whether or not they will infect *Agoseris grandiflora,* one of the hosts of *S. innominatum*. Until they have been studied carefully from living material and their complete life cycles and host range have been determined it is impossible to say how closely related they are. Nevertheless, they are similar to *S. innominatum* in being long-cycled, compositely dihomeogallic with predominantly deep-seated galls and by the development of their initial cells directly into sori of sporangia. Also, the sporangia are highly variable in size and shape with the walls thickened at the angles. In this respect, as well as in general appearance and life cycle, they are very similar to *S. taraxaci,* particularly the fungus on *Scorzonella laciniata*. Both Farlow and McMurphy regarded *S. innominatum* as being closely related to *S. taraxaci,* and it is possible that these two species and the three described above on other composite hosts constitute a group of closely related species. However, Ludi (**1902**) showed that *S. taraxaci* is limited in host range to few species of *Taraxacum* and will not infect other plants. Possibly, *Synchytrium innominatum* also has a limited host range, and further intensive host range studies may reveal the presence of biological races in these similar species. Obviously, such studies are needed to supplement morphological and developmental information on these species. It is to be noted that all of them have been reported only from California on fairly closely related composite hosts.

SUMMARY

Synchytrium innominatum is a long-cycled, compositely dihomeogallic species whose initial cell develops directly into a sorus of sporangia. Accordingly, so far as it is known, it appears to be a member of the subgenus *Eusynchytrium*. The predominantly deep-seated galls are formed usually by a combination of the inward enlargement and growth of the infected cell, inward elongation and division of the adjacent epidermal cells, and division of some of the palisade or mesophyll cells.

Three fairly similar long-cycled species which were previously determined as *S. innominatum* occur on *Microseris aphantocarpa*, *M. biglowii* and *Scorzonella laciniata* in California. These species are compositely dihomeogallic with largely-embedded or deep-seated galls, and their initial cell develops directly into a sorus of sporangia. Although they are similar in these respects to *S. innominatum* and occur on composite hosts also, it is not certain that they are identical with this species.

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AN UNDESCRIBED FUNGUS CAUSING A ROOT ROT OF STRAWBERRY

PAUL E. NELSON AND STEPHEN WILHELM

(WITH 2 FIGURES)

An imperfect fungus, apparently without characteristics of any previously described genus, which under California conditions has been proven to be pathogenic to roots of strawberry is described herein (8). Under field conditions, the fungus caused extensive root rot in one strawberry field and is believed to have been the primary etiologic agent responsible for the poor growth and ultimate failure of the planting. Under experimental greenhouse conditions, the fungus caused black, sunken lesions on the large, fleshy, adventitious roots, and on the branch roots, and killed numerous feeder rootlets. Large roots and rootlets were also killed from the tips back.

The disease caused by this fungus is a root rot, though it is readily admitted that other agencies, both in California and elsewhere, may cause the same type of disease. The fungus has shown pathogenic specificity for strawberry in greenhouse tests, but it can grow along the root surfaces, and possibly parasitize the roots of quite a number of other plants such as pepper, tomato, carrot, lettuce, and *Potentilla*, without causing any apparent injury.

Typically the fungus produces dark-brown chlamydospores both in culture and in the cortical tissues of affected roots (FIG. 1, A–C, H). These are so characteristic as to permit rapid identification of the root disease. In culture the chlamydospores are usually borne laterally on main hyphae and are either short-stalked or sessile. The conidial stage occurs readily in laboratory cultures on natural media sterilized with propylene oxide (2). Wheat and barley straws support excellent growth of the fungus and favor conidial as well as chlamydospore formation. The fungus rapidly loses its ability to sporulate when grown on potato dextrose agar. That the chlamydospore and conidial stages were produced by the same fungus was repeatedly demonstrated by initiating cultures from single spores.

The conidia are non-septate, hyaline, lunate with acuminate tips (FIG. 1, G). They are produced in dry heads (FIG. 1, E, F). The

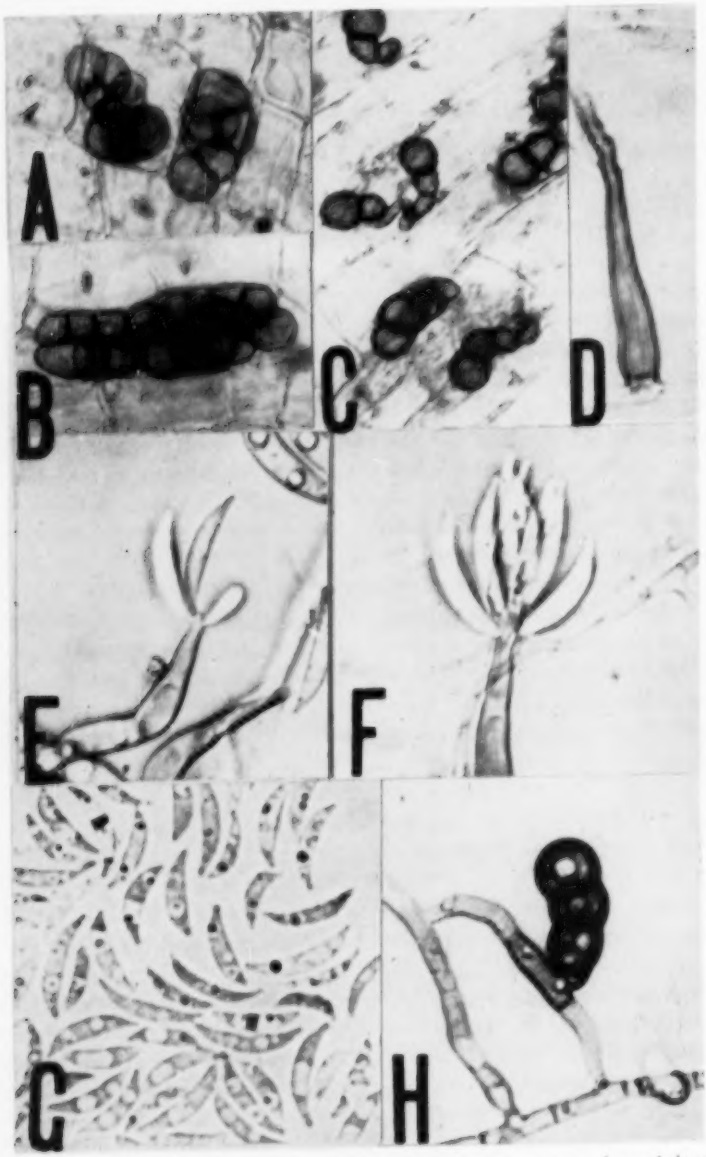

FIG. 1, A–H. *Idriella lunata*. A–C. Chlamydospore masses formed in the outer cortical tissues of strawberry roots. Two different isolates of the fungus

individual conidia are dry spores as defined by Mason (6) and by Wakefield and Bisby (7). The conidial heads are borne on short, distinct, light-brown, non-septate conidiohpores. The mature conidiophore is widest at the base and tapers toward the tip, commonly exhibits a distinct basal swelling, and may occasionally possess a basal septum. After the conidia have been shed, the conidiophore tip bears distinct spore scars, and appears somewhat geniculate (FIG. 1, D). The conidia begin development as small swellings on the tip of the conidiophore, and develop one at a time. As new conidia form on the elongating conidiophores, the mature conidia come to occupy a lateral position (FIG. 1, E).

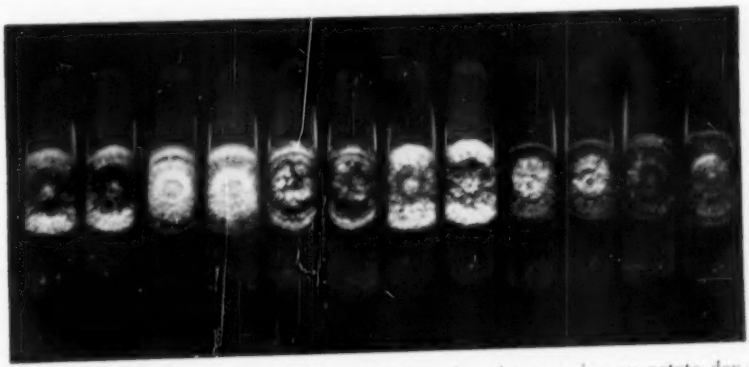

FIG. 2. *Idriella lunata*. 6 different isolates, in pairs, growing on potato dextrose agar. Cultures are 14 days old from single conidia, grown out of doors on a north window ledge.

The conidia typically remain in place until a head composed of 10 to 20 has been formed. The mycelium is hyaline at first but becomes dark with age. A perfect stage apparently is lacking. These characteristics indicate that this fungus is in the family Dematiaceae of the Moniliales of the Fungi Imperfecti. General colony characteristics are shown in FIG. 2.

The chlamydospores and conidiophores produced by this fungus suggest similarity or even relationship to *Ramularia* as emended by Wollenweber (9), particularly to those species described by Hildebrand (3) and Zinssmeister (10) as causing root rots of ginseng. However, the non-septate, distinctly lunate conidia borne in heads as contrasted with

are represented, × 500. D. Mature conidiophore showing conidial scars and geniculate tip, × 1500. E, F. Stages in the development of conidia and the dry conidial head, × 1250. G. Mature conidia, × 1500. H. Chlamydospore mass developing in culture, × 1250.

the short spore chains of *Ramularia* serve to distinguish it. *Acrotheca*, a similar genus, is considered by Hughes to be synonymous with *Ramularia* (**4**). A similarity to *Menispora* is suggested, more by Barnett's drawing of *M. cobaltina* (**1**), however, than by Persoon's original description. Corda's description of *Menispora* as bearing masses of hyaline, fusoid to falcate spores in mucus, and particularly his drawings (Icones Fungorum **2**, pl. 10, figs. 54–57) suggest that he may actually have observed a *Fusarium*. Hughes' characterization of the *Menispora* phialide as bearing a collarette formed by an early rupture of the wall (**5**) leaves little doubt that the fungus described herein could not be placed in *Menispora*. A genus having the characteristics of the strawberry root rot fungus apparently has not been described, and it becomes necessary to establish a new genus.[1]

Idriella gen. nov.: Hyphis hyalinis vel brunneis. Conidiophoris brunneis, simplicibus, non-septatis, nonnumquam 13–33 μ longis sed plerumque 16–26 μ, ad basim 3–5 μ crassis, bullatis, sursum attenuatis, geniculatis, conidia in capitibus siccis ferentibus. Conidiis lunatis vel falcatis, hyalinis, cum verticibus acuminatis. Chlamydosporis brunneofuscis, unicellularibus vel pluricellularibus, sessilibus vel stipitatis, hypharum lateribus natis.

Idriella lunata sp. nov.: Conidiophoris brunneis, non septatis, 13–33 μ longis, plerumque 16–26 μ, simplicibus, basi 3–5 μ crassa cum bullis distinctis, sursum attenuatis, geniculatis, conidiis in capitibus siccis ferentibus. Conidiis lunatis vel falcatis, hyalinis, 10–20 $\times$ 3–4 μ ad partem latissimam, cum verticibus acuminatis. Chlamydosporis brunneofuscis, unicellularibus aut pluricellularibus, sessilibus vel stipitatis, hypharum lateribus natis.

Hab. in radicibus vivis *Fragariae chiloensis* Duchesne var. *ananassae*.

The TYPE is represented by a single spore culture isolated originally from a diseased strawberry root from a field near Santa Clara, California, September, 1950. Material of this type has been placed in The American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Holland.

The name *Idriella* was chosen to represent this fungus because the conidiophores and conidial heads called to mind the smooth columnar trunk and terminal erect arm-like branches of the desert plant *Idria columnaris* Kellogg.

SUMMARY

An undescribed fungus causing root rot of strawberry is described and placed in a new genus, *Idriella*. The genus is characterized by the production in dry heads of non-septate, hyaline, lunate conidia,

[1] Latin descriptions were prepared by Mrs. Lily H. Davis, Department of Plant Pathology, University of California, Los Angeles, California.

on short, light-brown, non-septate conidiophores. Dark brown chlamydospores are produced in culture and in the cortical tissues of infected roots. The species has been designated *Idriella lunata*.

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SPOT ANTHRACNOSE OF LINDEN [1]

D. W. Creelman [2]

(with 1 figure)

In August, 1954, a destructive leaf-spotting disease was discovered on linden (*Tilia europaea* L.) at Lunenburg, Nova Scotia. Examination of the lesions revealed an abundance of ascomata bearing mature asci and ascospores which were recognized as belonging to the genus *Elsinoë* Racib. Subsequent observations showed the presence of lesions on petioles, ligulate bracts of fruits, and green twig growth. Infections were also found on another European species of linden, *T. platyphyllos* Scop.

NAME AND DISTRIBUTION

The disease appears to be new and undescribed. Its occurrence in Nova Scotia was first reported in the Canadian Plant Disease Survey, 1954 (**2**), under the name "anthracnose." It is proposed, however, to follow the suggestion of Jenkins (**1**) that the name "spot anthracnose" be used to designate diseases caused by *Elsinoë* and *Sphaceloma*. I have, accordingly, designated the disease on the linden "spot anthracnose of linden."

In 1955 the disease was again prevalent at Lunenburg. A survey of the lindens planted in twelve communities in western Nova Scotia revealed the presence of spot anthracnose at Bridgetown and Annapolis Royal. The Bridgetown outbreak was confined to eight trees of *T. europaea* imported from Holland in 1946. At Annapolis Royal, spot anthracnose was found affecting young trees of *T. europaea* in a nursery. These lindens were imported from Tennessee in 1955.

No species of *Tilia* are native to Nova Scotia, so it is apparent that spot anthracnose has been introduced with the trees. It is believed that the three outbreaks in the province represent three separate introductions of the disease.

While the diseased lindens at Bridgetown and Annapolis Royal are comparatively young, those at Lunenburg are many years old, with butt

[1] Contribution No. 1500 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Assistant Plant Pathologist.

diameters ranging from 1 to 3 feet. No recent introductions of linden have been found at Lunenburg. The disease here may have been present for some time and only noticed in 1954 because of the severity of the outbreak.

Since the discovery of the disease in Nova Scotia, evidence has become available to show that it is of wide distribution and possibly not rare. Dr. J. A. Lindquist had already found spot anthracnose on *T. europaea* in Argentina and collected representative specimens [3] in 1935. Fragments of these collections were communicated to the writer in 1955 by Dr. Anna E. Jenkins.[4] They exhibit lesions indistinguishable from those on linden in Nova Scotia. Acervular bodies, previously observed by Dr. Lindquist and seen by the writer, are identical in appearance with those of the *Elsinoë* on linden in Nova Scotia. No conidia or conidiophores were detected. Continued presence of the disease in Argentina is shown by a specimen collected by Dr. Lindquist in March, 1955.

A further distribution record of spot anthracnose has resulted from recent examinations of phanerogamic collections of *Tilia* deposited in several United States herbaria. These were examined by Dr. Jenkins. With permission of the curators concerned, she selected leaf fragments exhibiting lesions that might be the spot anthracnose. The writer compared them with specimens collected at Lunenburg. In this way a positive record was obtained of the disease on the North American species, *Tilia neglecta* Spach., in Virginia, 1938. As exemplified by the fragment selected, the lesions here are typical of the spot anthracnose. Moreover, ascomata of *Elsinoë* are present and these appear identical in all respects with those on the collections from Lunenburg.

SYMPTOMS

On the leaves the malady shows a particular affinity for the veins and the tissue immediately adjacent to them, although intervenal tissue is attacked as well (FIG. 1, *a*). The spots are at first black, extremely small, but gradually enlarge to 0.5–1.0 mm in diameter. They are circular, or mainly so, and have a definite, raised, black border. The centers of the spots become pale gray. As the spots age, the lower epidermis and mesophyll within the margins slough off, leaving the

[3] See specimens examined.

[4] The assistance of Dr. Anna E. Jenkins in obtaining collections of spot anthracnose from localities other than Nova Scotia, and her helpful suggestions in preparation of the manuscript, are hereby gratefully acknowledged.

paper-thin upper epidermis upon which may be seen the ascomata of the fungus (FIG. 1, *b*). Eventually, the upper epidermis also falls away leaving the leaves with a shot hole appearance. Where spots are numerous they may coalesce, and the appearance of the leaf late in the course of the disease is best described as ragged (FIG. 1, *c*). Spots on the ligulate bracts resemble those on the leaves (FIG. 1, *d*).

On the petioles, green twigs, and larger veins the lesions are elongate, decidedly concave, with a raised black border (FIG. 1, *e*). Spots on woody tissues tend to coalesce more readily than those on leaves, and

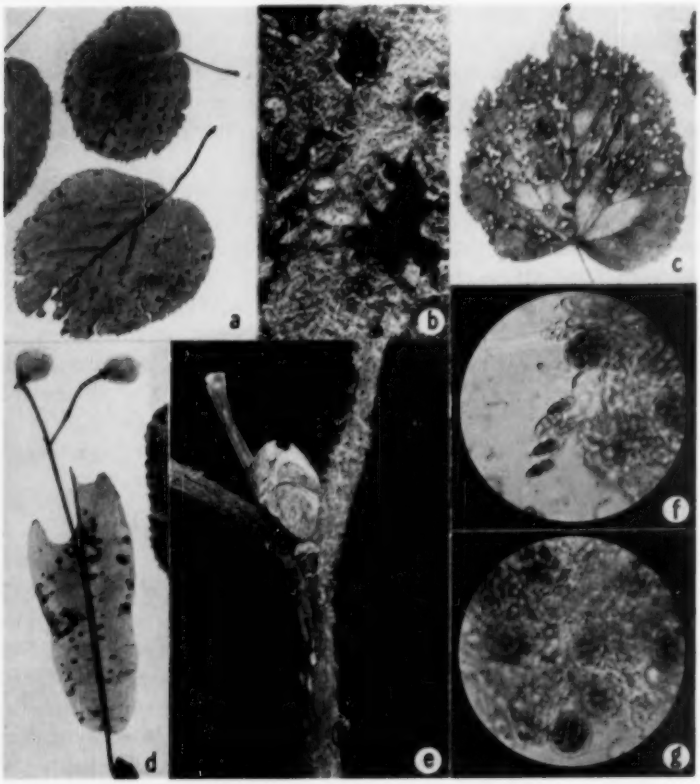

FIG. 1. *a*. Spot anthracnose lesions, showing distribution on leaf. *b*. Spots, showing ascomata, × 6.5. *c*. Advanced stage of disease on leaf. *d*. Lesions on bracts. *e*. Lesions on young twig, × 6.5 *f*. Asci and mature ascospores, × 600. *g*. Asci in crushed mount, × 600.

may result in lesions up to 10 mm in length and 2–3 mm in width. The epidermal tissue within the lesions turns gray and gradually curls upward, giving a scab-like effect. The scabs eventually fall away, leaving the affected parts with a pitted appearance.

DISEASE DEVELOPMENT AT LUNENBURG IN 1955

The first symptoms were observed on June 10, about two weeks after the trees had come into leaf. At that time the spots were few in number and were mostly confined to the marginal tissue of the apical portions of the leaves. On June 20, spots could be found on all parts of the leaf blade, and on petioles, bracts and green twig growth. On July 6, acervular bodies were observed in the older spots. These fruited in a moist chamber in the laboratory. They were recognized as the conidial stage (*Sphaceloma*) of the *Elsinoë*. Immature *Elsinoë* ascomata were seen on July 25, at which time all stages of the disease were in evidence. By mid-August the leaves were exhibiting the shot-hole appearance and were exceedingly ragged. Premature defoliation of the trees had begun.

Sphaceloma was isolated from lesions in the bud scales in April. This, in addition to the pattern of early infection, suggests that primary infection results from conidia produced on over-wintered lesions in the buds. Secondary infection could arise from rain-splashed spores from initial lesions on the new growth. New infections on sucker growth can be found following rains throughout the summer.

THE PATHOGEN

The fungus associated with linden spot anthracnose appears to be undescribed. A technical description follows.

Elsinoë tiliae sp. n.[5]

Maculae foliicolae atque in petiolis bracteis ramulisque, 0.5–1 mm diam., interdum coalescentes, pallide griseae, margine elevata cinctae. Ascomata epiphylla, superficialia, subspherica, 50–100 μ diam., coalescentia. Epithecia pallide brunnea. Asci irregulariter in stragulo uno vel duplici dispositi, pariete duplici, globosis vel ovatis vel pyriformibus, 8–15 in ascomate singulo, 19–25(–28) × 17–20(–23) μ, 8-spori. Spori obclavati hyalini, 3-septati, serius muriformes, 11–16 × (4.5–)5–6(–7) μ. Stromata conidialia acervularia, erumpentia, pulvinata, 30–50 × 10–20 μ crassa. Conidiophora acicularia, recta vel geniculata, continua. Conidia ovata, 3.7–6.5 × 1.8–2.5 μ.

[5] The Latin translation was kindly provided by Dr. Bernard Boivin.

Lesions numerous, on leaf blades, petioles, ligulate bracts of fruits and tender twig growth. Spots on leaves and bracts epiphyllous, small, 0.5–1.0 mm in diameter, black, becoming gray, with definite, raised black margins; mainly circular, visible below, distributed along veins and on intervenal tissue, frequently coalescing and involving areas up to 4 mm in diameter. Lesions on petioles and twigs elongate, concave, with raised black margins, centers of lesions drying and falling away, scab-like, through coalescence involving areas up to 15 × 5 mm.

Ascogenous stromata intraepidermal in origin, scanty; ascomata epiphyllous, superficial, subspherical, 50–100 μ in diameter, frequently aggregating into masses up to 500 μ in diameter. Asci irregularly distributed in one or two layers, surrounded by parenchymatous tissue composed of minute, hyaline cells; epithecium composed of a single layer of light brown, thick-walled cells. Asci double-walled, globose to ovate or pyriform, 8–15 in an ascoma, 19–25(–28) × 17–20(–23) μ, 8-spored; spores irregularly arranged, obclavate, hyaline, 3-septate, becoming muriform, 11–16 × (4.5–)5–6(–7) μ, mostly 13–14 × 5–7 μ, averaging 13.5 × 5.3 μ.

Conidial stromata intraepidermal in origin, epiphyllous, acervular, erumpent, numerous, scattered irregularly over surface of the spot or scab or sometimes coalescing, pulvinate, 30–50 μ in diameter, 10–20 μ thick, frequently sterile; conidiophores awl-shaped, straight to geniculate, continuous; conidia ovate, 3.7–6.5 × 1.8–2.5 μ, averaging 4.7 × 2 μ.

HOSTS: On living leaves, twigs and fruits of *Tilia europaea* L., *T. platyphyllos* Scop., and *T. neglecta* Spach.

SPECIMENS EXAMINED: On *Tilia europaea* L.: CANADA: Nova Scotia: Lunenburg, 1954, DAOM *48206*,[6] TYPE; Bridgetown, 1955, DAOM *48208*; ARGENTINA: Prov. Buenos Aires: Ciudad Eva Perón, 1935, IB *6187*, NFC *90741*; 1955, MIS *26200*, DAOM *48210*; Pilar, 1952, IB *6246*, NFC *90739*. On *T. platyphyllos* Scop.: CANADA: Nova Scotia: Lunenburg, 1954, DAOM *48207*. On *T. neglecta* Spach.: UNITED STATES: Virginia: Eastover, 1938, DAOM *48209*.

Paratype material deposited in the following herbaria: NFC, IB, MIS, and CMI.

[6] The following code letters are used to designate the various herbaria in which the specimens cited are deposited.

DAOM—Mycological Herbarium, Botany and Plant Pathology Division, Dept. of Agriculture, Ottawa, Ontario.

IB—Herbario da Seccao de Fitopatologia, Instituto Biologico, São Paulo, Brazil.

NFC—U. S. National Fungus Collection, Plant Industry Station, Beltsville, Md.

MIS—Colleciones Micologicas, Museo-Instituto Spegazzini, Ciudad Eva Perón, Argentina.

CMI—Commonwealth Mycological Institute, Kew, Surrey, England.

SUMMARY

Spot anthracnose of linden, discovered in Nova Scotia in 1954, is described as a new disease. A description of the symptoms on leaves, bracts and twigs is given, and a description of the pathogen, a hitherto undescribed species of *Elsinoë* is offered. Based on specimens examined, distribution of the disease in Nova Scotia, Virginia, and Argentina is shown.

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THE IDENTIFICATION OF THE CURVULARIA PARASITE OF GLADIOLUS [1]

J. A. Parmelee [2]

(with 9 figures)

In 1948 Dr. R. O. Magie first reported the incidence of *Curvularia* as the cause of a disease of gladiolus. The pathogen was tentatively identified at Beltsville, Maryland, as being similar to or identical with *Curvularia lunata* (Wakker) Boedijn.

When the first Canadian occurrence of *Curvularia* on gladiolus was reported (Parmelee, **1954**), no specific epithet was applied to the parasite. This was because the fungus seemed to fit the descriptions of *C. lunata* and *C. trifolii* (Kauffm.) Boedijn equally well and because difficulty was experienced in distinguishing these species. Pathologists currently use the binomial *C. lunata* for the gladiolus parasite. The purpose of this paper is to consider the morphological distinctions between *C. lunata* and *C. trifolii*, to establish the nature of the hilum as the distinctive character and to show that the *Curvularia* pathogen causing leaf, stem and flower spot and corm rot of gladiolus agrees more closely with *C. trifolii*.

Curvularia lunata (Wakker) Boedijn (Figs. 1–3)

Wakker (**1898**) described *Acrothecium lunatum* from dead leaves of sugar cane grown in Java. His description, which is quoted verbatim in Saccardo's *Sylloge Fungorum* **14**: 1089, does not mention the hilum or point of attachment of the spore.

A. lunatum was chosen by Boedijn (**1933**) as the type species of his genus *Curvularia*, which included some species of *Acrothecium* among other phragmosporous Dematiaceae. A translation of Boedijn's description follows:

"Mycelium septate, profusely branched, in the substratum subhyaline to light brown, at the surface brown; individual threads 2–5 μ in diameter. Conidiophores dark brown, unbranched, septate toward the tip,

[1] Contribution No. 1506 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Assistant mycologist.

sometimes twisted, 70–270 × 2–4 μ. Conidia brown, in groups of a few, terminal, arranged in several whorls one on top of the other, or more or less spiral, rowboat-shaped, rounded off toward the tip, usually slightly attenuated toward the base, with three partitions, the second cell from the top much larger and darker colored than the others, while on this cell the conidium is retroflexed: 19–30 × 8–16 μ (mostly 23 × 11 μ)."

Accompanying figures of the spores do not illustrate clearly the hilum or point of attachment to the conidiophore. Reference is made to this character, however, under *Curvularia andropogonis* (Zimmerman) Boedijn. "Instead of the dark ring-shaped scar, one finds at the place of attachment a small hyaline appendage, a characteristic not occurring in any of the other species." This implies that a protruding hilum was not present in *C. lunata*. It was present in only one of the species that Boedijn examined (*C. andropogonis*).

It is now known that *C. trifolii* also has a protruding hilum but Boedijn had not examined *Brachysporium trifolii* Kauff. He had simply made the combination *Curvularia trifolii* (Kauff.) Boedijn using the characters given by Bonar (**1920**). In Bonar's paper Kauffman, who supplied the technical description, made no reference to the hilum. He described the spores as "obtuse to rounded above, subacute downwards."

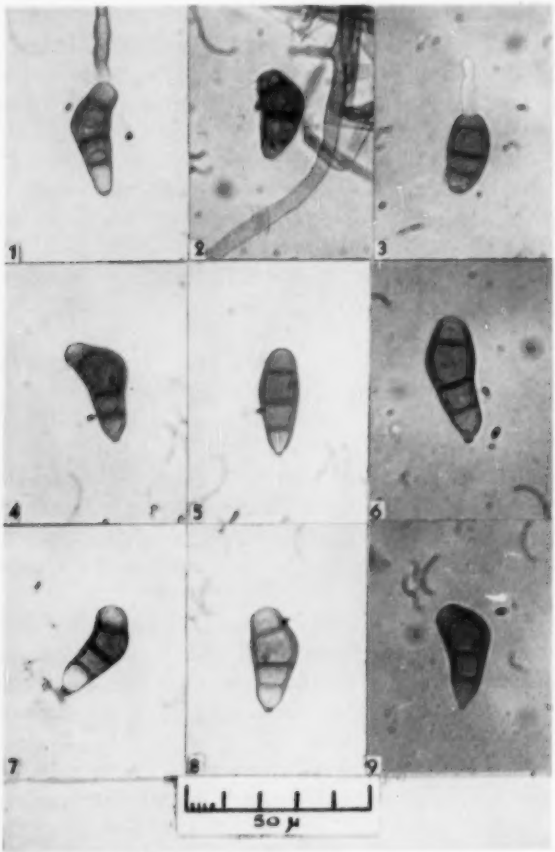
Groves and Skolko (**1945**) referred a number of seed-borne isolates to *Curvularia lunata*. The spore dimensions they recorded correspond reasonably well with those given by Boedijn. Again the character of the hilum was not considered, but in subsequent studies of their material I found it to be inserted.

Subramanian (**1953**) described *C. lunata* from dead stems and leaves of cultivated grasses and palms. His clear outline drawings of the spores illustrate an inserted hilum and he refers to a "somewhat obconical basal cell which bears a scar indicating the point of attachment to the conidiophore."

A culture of *C. lunata* originally isolated by Boedijn and maintained at the Centraalbureau voor Schimmelcultures, Baarn, Holland, was examined. A few spores were seen but conidiophores were not observed. The spores were more elongated (TABLE I) than those measured by either Boedijn (**1933**) or Groves and Skolko (**1945**).

Various attempts have been made by Dr. E. S. Luttrell of the Georgia Experiment Station and by the writer to obtain information from Bogor, Indonesia, and from Leiden, Holland, regarding the disposition of Wakker's type. From correspondence with Dr. M. A. Donk,

formerly Keeper, Herbarium Bogoriense, now retired, we have concluded that the type is not in existence. It will thus become necessary to choose a neotype but it should preferably be based on material on sugar cane from the type locality and attempts to obtain such material have so far been unsuccessful.



FIGS. 1–3. *Curvularia lunata*. 1. IMI *22812* on Dosooti, Pakistan. 2. DAOM *27451* on *Saccharum munja*, India. 3. Luttrell *1004* on *Saccharum officinalis*, Florida. 4–6. *Curvularia trifolii*. 4. Luttrell *945* on *Trifolium repens*, Alabama. 5. Luttrell *781* on *T. repens*, Georgia. 6. DAOM *14182* (part of TYPE), Maryland. 7–9. *Curvularia trifolii* f. sp. *gladioli*. 7. Magie *20* on var. Picardy, Florida. 8. DAOM *48146* on var. Gaylore, Ontario. 9. DAOM *48145*, on plants from cormel stock, Florida.

TABLE I

CURVULARIA LUNATA (WAKKER) BOEDIJN

Measurements by	Spores		L/W	Hilum	Septa	Mounting medium	Substrate
	Length	Width					
Wakker	30	10		—	3–4	—	dead leaves
Boedijn (1933)	19–30	8–16	2.0	inserted	3	—	sugar cane air, *Ricinus*, *Mangifera*
Groves and Skolko (1945)	(19.5)22–28(32.0)	(9)10–13(15.5)	2.1	inserted*	3	lactophenol	*Allium* *Capsicum* } seed
Subramanian (1953)	17–24	8–12	2.0	inserted	3	—	dead leaves *Marina* *Borassus* *Eleusine*
Parmelee	21–35.8	8.6–11.4	2.8	inserted	3	lactophenol	Boedijn culture
Parmelee	22.2–30.0	11.4–16.4	1.8	inserted	3	lactophenol	sugar cane
Parmelee	20.0–32.9	9.3–15.7	2.1	inserted	3	lactophenol	**

* Denotes writer's observation.

** Observations on six specimens: DAOM *19456* Tent, New Guinea; DAOM *15773* Tarpaulin, Panama Canal Zone; DAOM *19455* Canvas, Fla.; IMI *22812* Dosooti, Pakistan.

TABLE I compares spore size, the ratio of length to width, and hilum character of *C. lunata* as determined by various writers. Boedijn's measurements and observations are basic for the correct concept of this species.

CURVULARIA TRIFOLII (KAUFFMAN) BOEDIJN (FIGS. 4–6)

The spores of *Brachysporium trifolii* were described by Kauffman as follows: "unequally ventricose-pyriform, mostly curved, varying to obovate, very rarely triangular-lobed, obtuse to rounded above, subacute downwards, usually 3-septate, antepenultimate cell enlarged especially on the convex side, dark fuscous brown, thin-walled and smooth 21–31 × 9–11 μ."

In this description the hilum is not mentioned. Although Groves and Skolko (**1945**) in their Pl. II, fig. 12, illustrated the protruding hilum in spore drawings from type material, they omitted this character in their description of the species. In distinguishing between *C. lunata* and *C. trifolii* they emphasized spore size. Their measurements of spores (TABLE II) obtained from type material ranged somewhat larger than the dimensions given by Kauffman. Spore size determined by the writer from part of the type agrees with that given by Groves and Skolko whereas Luttrell's findings (in litt.) approximate those of Kauffman. Luttrell's studies were done with water mounts of the fungus. It would be tempting to ascribe this discrepancy in size to the greater swelling action of lactophenol on the spore, but when the writer compared mounts in water and lactophenol both from type material and from Luttrell's isolate No. *781* from *Trifolium repens* this size difference was not apparent. Personal error, difference between individual spore mounts and to some extent the kind of mounting medium must all slightly affect the size ranges obtained.

Luttrell (in litt.) drew the writer's attention to the protruding hilum in *C. trifolii*. This character is evident in the type and in a number of *Curvularia* isolates from ladino clover and is a consistent morphological distinction between *C. lunata* and *C. trifolii*.

The seed-borne isolates from *Glycine max* (L.) Merr. (soybean) and *Pisum sativum* L. (peas) produced in Ontario and from *Cucumis sativus* L. (cucumber) from New Jersey and from *Cucurbita pepo* L. (pumpkin) from Connecticut that were identified as *C. trifolii* by Groves and Skolko were all re-examined. The spore size in these isolates was generally greater than that found in *C. lunata* but there was considerable overlapping in size ranges. For instance spores isolated from peas (DAOM *14245*) were 24.3–30.0 × 10.7–15.7 μ, whereas spores from

TABLE II

CURVULARIA TRIFOLII (KAUFF.) BOEDIJN

Measurements by	Spores		L/W	Hilum	Septa	Mounting medium	Substrate
	Length	Width					
Kauffman (1920)	21–31	9–11	—	—	3	—	*T. repens*
Groves and Skolko (1945)	25.0–35.0(38.0)	11–15	2.3	[protruding]	3	lactophenol	TYPE
Parmelee	(24.3)26.4–38.6(40.0)	10.0–17.2	2.4	protruding	3(4)	lactophenol	TYPE
Luttrell	22.4–33.6	8.4–14.0	2.5	protruding	—	water	TYPE
Luttrell (Luttrell *781*)	22.4–32.2	8.0–10.6	2.9	protruding	—	water	*T. repens*
Parmelee (Luttrell *781*)	25.7–37.2(42.9)	10.0–12.9	2.7	protruding	3(4)	lactophenol	*T. repens*

TABLE III

CURVULARIA TRIFOLII F. SP. GLADIOLI

Locality	Measurements by	Spores		L/W	Hilum	Septa	Mount medium
		Length	Width				
Philippines	Mendiola-Ela (1952)	(18.5)20.0–32.9(36.4)	8.5–14.4(16.4)	2.3	protruding	3	—
Oregon	Anderson *et al* (1952)	24.0–30.0	8–12	2.7	—	3	—
Eastern Can. & U.S.A.	Parmelee	20.0–38.6	8.6–18.6	2.1	protruding	3(4)	lactophenol
East. Can.	Parmelee	25.7–38.6	12.1–18.6	2.0	protruding	3	lactophenol
East. Can.	Luttrell	22.4–37.8	11.2–15.4	2.1	protruding	3	water

soybean (DAOM *14239*) were 21.5–36.5 × 11.4–19.3 μ. In a second collection from peas (DAOM *15110*) the observed size range was 22.9–35.8 × 10.7–18.6 μ. The most striking difference when compared with the type of *C. trifolii* was to be found in the hilum, which is strongly protruding in the latter, but not at all in these isolates. When this morphological difference is considered, it is obvious that these specimens do not belong in *C. trifolii*. Their redisposition is postponed until such time as a complete study is made of the entire *lunata* group.

Subramanian (**1953**) described and illustrated a collection of *Curvularia trifolii* from dead culms of *Scirpus* (Herb. MUBL No. *851*—I). Identification was based on the redescription by Groves and Skolko of *C. trifolii*, which stresses spore size. Subramanian acknowledged that his fungus occurred on a different host from that on which the species was originally described but he attached little importance to this because species of *Curvularia* had been reported on many diverse substrates. His fig. 5 clearly illustrates spores with inserted attachment scars. Because *C. trifolii* has a protruding hilum this record is obviously a misdetermination, even though the spore size may be similar to that of the clover fungus. The disposition of this record is not attempted at this time.

When spore measurements are compared from TABLE I and TABLE II, size in the latter is seen to be somewhat larger than in the former. Length and width figures obtained by Kauffman and Luttrell (TABLE II) do not substantiate this observation, however, and their results agree very closely with spore size given in TABLE I. It is obvious then that spore size alone is not a good standard for separating *C. trifolii* and *C. lunata*. The principal morphological distinction is in the character of the hilum.

THE CURVULARIA PATHOGEN OF GLADIOLUS

As stated at the beginning of this paper, the *Curvularia* isolated by Magie from gladiolus was originally identified as *C. lunata*. Succeeding reports have all taken up this binomial. Mendiola-Ela (**1952**) reported the *Curvularia* disease of gladiolus from the Philippines. After extensive studies of spore size, she concluded that the pathogen was *C. lunata*. Her results are listed in TABLE III. They compare well with *C. lunata* (TABLE I) but she refers to and illustrates a protruding hilum. It is readily seen how the fungus may be identified as *C. lunata* when the character of the hilum is not considered. However, it will be seen that the measurements listed by Miss Mendiola-Ela agree just as well with those for some specimens in TABLE IV, for example, those from var.

TABLE IV

CURVULARIA TRIFOLII F. SP. GLADIOLI

DAOM No.	Glad. var.	Location	Spores		L/W	Hilum	Septa	Substrate
			Length	Width				
40227	Radiance	Que.	22.9–37.2	8.6–14.3	2.6	protruding	3(4)	PDA
	—	Que.	25.7–35.8(40.0)	10.0–15.7	2.3	protruding	3(4)	corms
	Harry Hopkins	Que.	20.0–34.3	8.6–16.4	2.1	protruding	3(4)	PDA
	—	Que.	21.5–32.9(35.8)	10.7–17.2	1.9	protruding	3	corm scales
	Spic & Span	Que.	22.9–32.9	10.7–14.3	2.2	protruding	3(4)	PDA
	Aotearoa	Que.	24.3–35.8	9.3–16.4	2.3	protruding	3(4)	PDA
	Pactolus	Que.	25.7–35.8	10.7–15.7	2.32	protruding	3	PDA
	Lipstick	Que.	(21.5)25.7–34.3	9.3–14.3	2.47	protruding	3	PDA
48235	—	Que.	25.7–34.3	12.9–16.4	2.04	protruding	3	leaves
48236	Lake	Ont.	25.7–38.6	12.1–18.6	2.09	protruding	3	leaves
48146	Gaylore	Ont.	25.7–34.3	12.1–18.6	1.94	protruding	3	leaves
48240	Leading Lady	Fla.	(20.0)25.7–35.8	(8.6)10.7–13.6(15.0)	2.60	protruding	3(4)	PDA
48241	Picardy	Fla.	24.3–34.3	10.0–14.3	2.41	protruding	3(4)	PDA
48145	—	Fla.	21.5–30.7	9.3–15.7	2.09	protruding	3	leaves
48237	—	N.Y.	24.3–33.6(37.2)	10.0–14.3	2.38	protruding	3(4)	PDA

Harry Hopkins, Spic and Span, or DAOM *48145*. The writer's observations of the gladiolus isolate are summarized in TABLES III and IV. In general, spore size compares more favorably with that of *C. trifolii* although, as shown in TABLE IV, spore size is not constant between collections.

It might be noted that, previous to the writer's observations on this fungus, Dr. J. W. Groves had linked the gladiolus pathogen with *C. trifolii*. His identification had been based on spore size alone. In 1948, in answer to a letter from Dr. F. P. McWhorter of the Oregon Experimental Station, he had written as follows: "This fungus seems closer to *Curvularia trifolii* than to *C. lunata*. In all the characters I use to distinguish these two forms your pictures exhibit those of *C. trifolii*. The measurements were 23–38 × (10)12–17 μ and this is definitely out of the range I would put in *C. lunata*. As far as I can tell from the photographs it matches the type of *C. trifolii* (Kauffman) Boedijn."

Spore size is not conclusive, but the protruding hilum definitely associates the gladiolus *Curvularia* with *C. trifolii*.

INFECTION STUDIES

If the isolate from gladiolus is typical of *C. trifolii* one would expect it to be similar biologically as well as morphologically.

Six isolates from gladiolus were sprayed on *Gladiolus* and *Trifolium repens*. The hosts were held in incubation chambers for two days, and within one week all the gladiolus plants showed severe leaf spotting. Clover showed no evidence of susceptibility. A similar test using a clover isolate (Luttrell No. *945*) was carried out at the same time. Neither host showed a positive reaction. In a previous test of three clover isolates only the Luttrell No. *945* had resulted in infection of Ladino clover.

All experiments in which the corms were inoculated by a stab-wounding method have given positive results with gladiolus isolates and negative results with clover isolates.

The evidence indicates that the clover and gladiolus fungi are biologically distinct; yet, it is difficult to say positively that the gladiolus fungus is not capable of infecting clover when the results with clover isolates on clover have been so inconclusive. However, if we accept the existing evidence that these fungi are biologically distinct and morphologically identical, then it is considered that the gladiolus fungus should be distinguished as a special form of *C. trifolii*, *C. trifolii* (Kauffm.) Boed. f. sp. **gladioli** Parmelee and Luttrell.

SUMMARY

Formerly *C. lunata* and *C. trifolii* have been separated on differences in spore size. This has led to a misconception of *C. trifolii* and consequently misdeterminations have resulted.

The hilum, which is protruding in *C. trifolii* and inserted in *C. lunata*, is the most reliable character on which these species can be separated.

ACKNOWLEDGMENTS

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HAPLOSPORANGIUM IN SASKATCHEWAN RODENTS [1]

A. Bakerspigel [2]

(with 2 figures)

Most of the reports on the pulmonary fungus *Haplosporangium parvum*, found in rodents and other animals, have come from the United States. Emmons and Ashburn, in 1942 (**5**), cultured this fungus from the lungs of various rodents trapped in southern Arizona. Emmons (**4**) proposed the term "haplomycosis" for this type of infection in the rodent lungs. Erickson (**6**) noted that the large spherules in the lungs of a beaver, trapped in 1941 in Minnesota, were identical with those of *H. parvum*. Later, in 1950, Jellison (**7**) reported *Haplosporangium* not only in beaver but also in muskrat, pine squirrel and other animals.

In England, McDiarmid and Austwick (**9**) recorded epizootics in moles that occurred in 1947 and in 1953 on the Berkshire downs. Upon histological examination of the lungs they found large, thick-walled spherules 100–200 μ in diameter. Though fresh material from these epizootics was not available, they succeeded in isolating *H. parvum* in culture from other moles trapped in the Exeter district.[3] More recently, Jellison (**8**) reported finding *Haplosporangium* in Korean rodents. However, this organism did not seem to have any relationship to cases of hemorrhagic fever in humans in Korea. In a personal communication from Dr. William Carmichael, University of Alberta, the author was informed that *Haplosporangium* had also been found by Dr. R. Ciferri in Italy.

In Canada, Dowding (**3**) was the first to report *Haplosporangium* in Alberta rodents and to study its relationship with *Blastomyces dermatitidis, Histoplasma capsulatum* and *Coccidioides immitis*. Dowding concluded that *Haplosporangium* is a Phycomycete closely resembling *Chaetocladium* and that all of these fungi constituted a natural group. Later, in the same laboratory, Carmichael (**2**) studying the strains and

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[3] *H. parvum* has recently been reported in three additional mammals in Britain by L. Tevis (Nature **177**: 437. 1956).

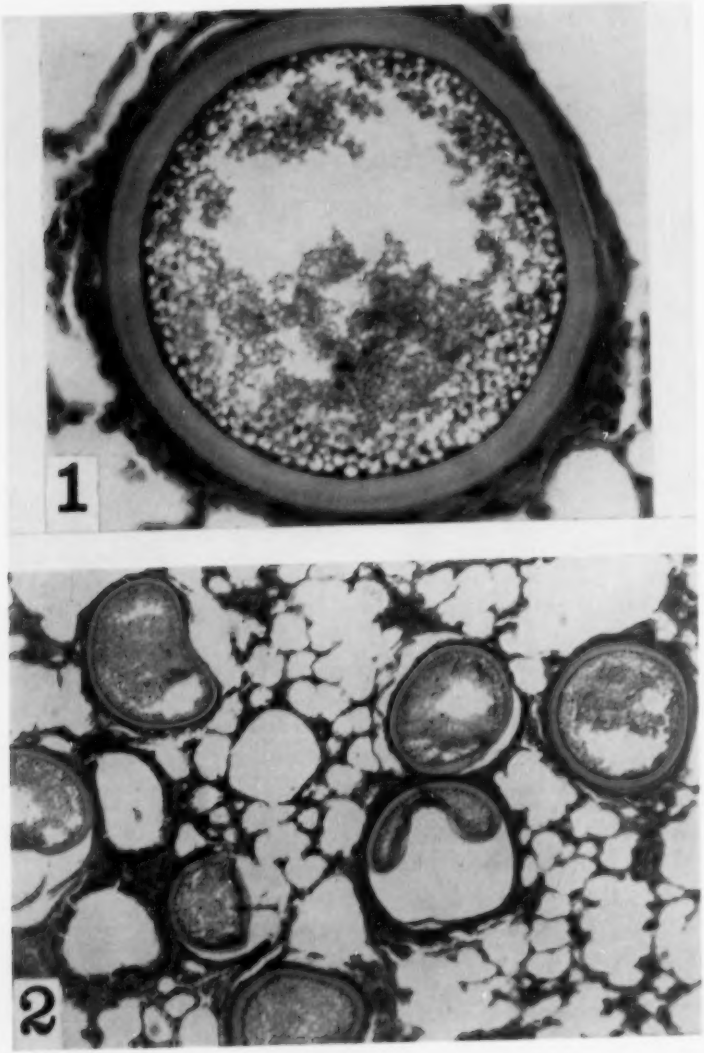
generic relationships of *Haplosporangium*, concluded that this fungus is probably not related to *C. immitis* and that its closest affinity is with *B. dermatitidis*. It is interesting to note that Breslau (**1**) recently showed the walls of the spherules of *Haplosporangium* to be composed of cellulose lying in a protein matrix relatively free of phospholipids. He concluded from this and other evidence that *Haplosporangium* is not related taxonomically to *C. immitis*, since the walls of the spherules of *Coccidioides* contain chitin lying in a protein matrix having an exterior layer of phospholipid.

In the light of the results obtained in Alberta, a survey was begun in Saskatchewan during the summer of 1955 with the purpose of isolating *Haplosporangium* and other fungi in the rodent population. Rodents were trapped from Saskatoon in the south to Big River in the north and from Lloydminster on the Alberta border in the west to the Manitoba border in the east. The results obtained during this survey in Saskatchewan are being reported to extend the recorded distribution of *Haplosporangium* in Canada.

260 rodents were trapped from May to September, 1955. They comprised the following animals: Beaver (*Castor*) 1; Chipmunk (*Eutamius*) 9; Squirrel (*Glaucomys*) 1; Rabbit (*Lepus*) 1; Meadow vole (*Microtus*) 88; Mouse (*Mus*) 2; Pocket mouse (*Perognathus*) 10; White-footed mouse (*Peromyscus*) 138; Rat (*Rattus*) 1; Shrew (*Sorex*) 2; Pocket gopher (*Thomomys*) 4; Jumping mouse (*Zapus*) 3.

Upon autopsy, 31 animals showed various signs of gross pathology in the lungs and/or liver ranging from small pearl-grey nodules on the lungs to confluent yellow or brownish patches on the lobes and edges of the liver. When sections of these organs were stained with eosin and hematoxylin and examined microscopically, only those from the lungs of 2-white-footed mice (*Peromyscus maniculatus borealis*) showed heavy infection with the spherules of *Haplosporangium*. The sections from the other 29 specimens of lungs and livers (from 12 white-footed mice and 17 voles) showed infestation with parasites other than *Haplosporangium*, such as nematodes. The 2 white-footed mice with spherules in their lungs had been trapped in a wooded area near the south Saskatchewan River, on the outskirts of Saskatoon. Their lungs were covered with numerous small pearl-grey nodules 0.5–1.0 mm in diameter. The nodules appeared similar to those previously described and illustrated by Erickson (**6**), Jellison (**7**) and Dowding (**3**).

FIGS. 1 and 2 are photomicrographs of lung sections, stained with eosin and hematoxylin, from a white-footed mouse (*Peromyscus* No. 8) which shows the unusually heavy infection of the lungs with the thick-



FIGS. 1, 2. Spherules of *Haplosporangium* in lungs of Peromyscus, No. 8. 1. Enlargement of a spherule, × 750. 2. Heavy infection of lungs with spherules, × 215.

walled spherules of *Haplosporangium*. These spherules vary from 79.2–198 μ in diameter, larger than the Arizona strain, and comparable in size with the Alberta strains described by Dowding (**3**). The walls of these spherules measure from 11–13.2 μ in thickness and appear to be composed of two layers—an outer thinner layer and an inner thicker one. Though FIG. 2 shows a large number of spherules in the alveoli of the lungs, no definite granulomatous tissue was noted around these spherules. The interior of the spherules appears to be composed of small globules, shown by Breslau (**1**) to be acid mucopolysaccharide.

Infected material from the lungs of these mice was not available for culture since they had been received fixed in formalin. Therefore the identification of the strain of *Haplosporangium* in Saskatchewan rodents and a comparison with the Alberta and American strains will have to await additional isolations during future surveys.

SUMMARY

The lungs of 2 white-footed mice (*Peromyscus maniculatus borealis*) trapped during June, 1955, near Saskatoon, were found to be heavily infected with the spherules of the pulmonary fungus, *Haplosporangium*. These mice were trapped during a survey, one phase of which was to detect mycotic infections in Saskatchewan animals. This finding constitutes the first report of *Haplosporangium* in Saskatchewan rodents and extends the recorded distribution of this fungus in Canada.

ACKNOWLEDGMENTS

The author wishes to thank Dr. E. S. Dowding (Mrs. Keeping) of the University of Alberta for examining representative lung sections and for her opinion concerning the infections in the mice.

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SOME FUNGAL PARASITES OF PORTULACACEAE [1]

D. B. O. Savile and J. A. Parmelee [2]

(with 3 figures)

A number of new records have recently come to hand of fungi attacking Portulacaceae in British Columbia, Ontario and Quebec. These records are here presented, together with other records and revisions necessary to an understanding of the parasites recorded in Canada. Six new taxa are herein named and another is reported but not named.

UREDINALES

Puccinia mariae-wilsoniae G. W. Clint. in Peck, Bull. Buffalo Soc. Nat. Sci. **1**: 66. 1873, var. **mariae-wilsoniae.**

According to recommendations 82 C and 82 D of the International Code (**5**) Clinton's epithet *mariae-wilsoni* should be regarded as an orthographic error, since it is recommended that the letter *i* be inserted after a name ending in a consonant other than *r* and that the epithet be in feminine form when derived from a woman's name.

This familiar rust occurs plentifully on *Claytonia virginica* in southern Ontario (Essex to Niagara Peninsulas) where this host is common. Elsewhere in the beech-maple forests of southern Quebec and Ontario it occurs commonly on the widespread *C. caroliniana*. In Gatineau Co., Quebec, it has been traced to Lac Philippe, some 15 mi. NW of Ottawa. Here the beech-maple begins to give way to mixed forest and *Claytonia* becomes very scarce. It thus appears that in eastern Ontario and western Quebec *P. mariae-wilsoniae* is virtually co-extensive with its host, in contrast with the known distribution of the other two rusts attacking this plant. An amplified description follows.

Pycnia chiefly epiphyllous. Aecia amphigenous, often diffuse and infections often numerous because plants heavily bombarded by basidiospores as they grow up through forest litter, hence sometimes appearing

[1] Contribution No. 1495 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Senior Mycologist and Assistant Mycologist, respectively.

to be systemic although never actually so; cupulate with conspicuous peridium (see FIG. 1). Aeciospores 18–27(–29) × 14–20 μ; wall 0.5–1.0 μ, hyaline, closely and minutely verrucose; germ-pores invisible. Uredinia wanting. Telia scattered, amphigenous, ca. 0.1–1.0 mm diam., dark brown. Teliospores (30–)33–56 × 16.5–27 μ, very slightly or not constricted at septum; wall 1.5–2.5(–2.9) μ thick, yellow-brown to light chestnut, evenly verrucose with rounded warts 0.2–0.5 μ high, 0.5–1.5 μ wide, 1.4–3.0 μ spacing between centers; germ-pores apical and ½–⅞ depressed, each with shallow, pale papilla; pedicel hyaline, deciduous.

PUCCINIA MARIAE-WILSONIAE G. W. Clint. var. **montiae** Savile var. nov.

A typo differt teliosporis brevioribus latioribusque, 27–46(–48) × 20–28.5 μ; pariete minute crassiore, 1.6–3.0 μ, verrucis 0.9–1.5 μ lat.

On *Montia sibirica* (L.) Howell, Skamania Co., Wash., W. N. Suksdorf (Barth. N. A. Ured. *133* in DAOM, **TYPE**); Big Cottonwood Canyon, Salt Lake Co., Utah, A. O. Garrett (Fungi Utahenses *67* in PUR). On *M. cordifolia* (S. Wats.) Pax. & K. Hoffm., Box Camp Meadow, Trinity Summit, Humbolt Co., Calif., J. P. Tracy *18445* (PUR ex UC). The Arthur Herbarium contains two other packets, from different sources, of the type collection, in addition to N. A. Ured. *133;* and also an aecial collection by Garrett on the same host, which is too overmature to provide any comparison with the eastern rust. The specimen on *Claytonia megarrhiza* on which Arthur (1) based the record of *P. mariae-wilsoniae* on this plant is *P. agnita* (q.v.). This rust thus seems to be restricted to *Montia sibirica* (= *M. asarifolia, Limnia alsinoides,* etc.) and the closely related *M. cordifolia.* Accordingly, although its range is distinct from that of the eastern rust on *Claytonia,* it seems advisable to treat it as a variety (in the sense widely accepted by uredinologists for a host-specialized variant) rather than as a geographic subspecies.[3]

[3] After this report was completed we noticed the record by Trelease (in P. A. Saccardo, C. H. Peck and W. Trelease. The fungi of Alaska. Harriman Alaska Expedition, part 5: 38. 1904) of this species, under the name *Aecidium claytonianum* (Schweinitz) Clinton, from St. Paul I., on *Claytonia arctica.* Arthur, who cited other records from the Harriman report and saw many of Trelease's specimens, did not cite this one and left no notes concerning it in the Arthur Herbarium. We are obliged to Dr. Robert E. Woodson, Jr., for searching the Harriman collections at Missouri Botanical Garden. He was unable to find this *Aecidium* or any specimen of *Claytonia arctica* from St. Paul I. Unless evidence is forthcoming to the contrary, it thus seems best to assume that the record is based on an error in host determination and that some unrelated rust was involved. The known distribution of *Puccinia mariae-wilsoniae* renders its occurrence in Alaska unlikely.

PUCCINIA AGNITA Arth., Bull. Torr. Bot. Club **42**: 590. 1915.

Pycnia in small groups, amphigenous. Aecia and uredinia lacking. Telia in circular or elliptical groups 2–7 mm diam., centered about pycnia, mainly hyphophyllous. Teliospores 30.5–48 × 19–30(–32) μ, not or very slightly constricted; wall 1.4–2.8 μ thick, yellow-brown to light chestnut, regularly verrucose with rounded warts 0.2–0.7 μ high, 0.6–1.5 μ wide, 1.4–3.0 μ spacing. Germ-pores ± apical and ½–¾ depressed, with small, pale papillae. Pedicel hyaline, deciduous.

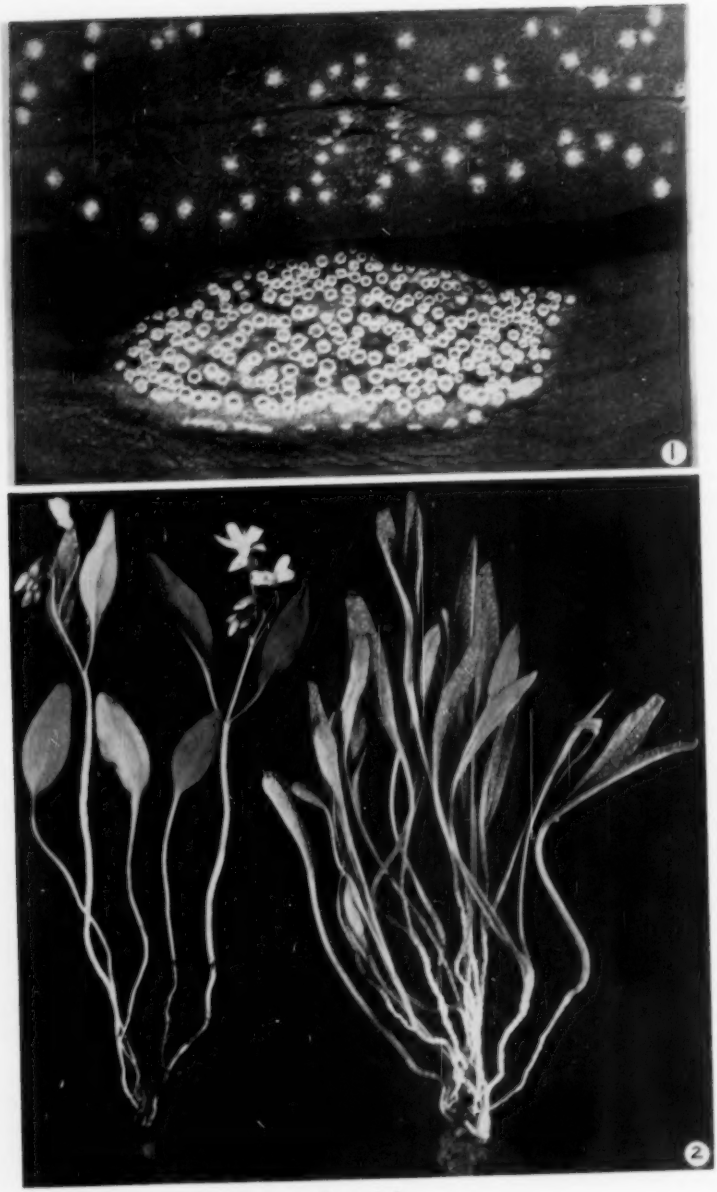
On *Claytonia megarrhiza* (Gray) Parry, Cumberland Mine, La Plata Mts., Colo., C. F. Baker, F. S. Earle and S. M. Tracy (Plants of Southern Colo. *1045*, in PUR, **TYPE**); James Peak, nr. Tolland, Boulder Co., Colo., E. Bethel (PUR *35635*). In the latter specimen, which is the source of Arthur's record of *Puccinia mariae-wilsoniae* on this host, no pycnia can be seen; but their apparent absence may be due to the specimen's having been taken in late summer when, after profuse sporulation, the teliospores covered the entire sorus. The wall markings are slightly less conspicuous than in the type, but in other respects agreement is good. The grouping of the telia clearly shows that the rust is microcyclic. It may be noted that both specimens are from 12,000 ft., which may account for the paucity of records of this rust.

UROMYCES CLAYTONIAE G. W. Clint. & Peck, Ann. Rep. N. Y. State Mus. **29**: 50. 1878.

The type specimen in the New York State Museum consists of two leaves bearing spent aecia and a scattering of small telia. From this specimen the following description has been drawn up, which is probably incomplete in respect to aeciospore size range.

Pycnia apparently amphigenous. Aecia amphigenous, in groups, cupulate. Aeciospores (few seen) 24–27.5 × 22–24.5 μ; wall ca. 1.0–2.5 μ thick, pale brownish yellow (from age?), moderately finely and patchily verrucose; germ-pores barely visible, apparently several. Telia scattered, amphigenous, less than 1 mm diam. Teliospores 30–53 × 19–28.5 μ; wall 1.3–3.2 μ thick, pale yellow to light chestnut, nearly smooth to sparingly and shallowly ridged or warted; germ-pore with slight to conspicuous hyaline papilla 1.0–4.0 μ high; pedicel ± hyaline, deciduous or weakly persistent.

On *Claytonia caroliniana* Michx. Known only from the type area, Cold Spring, Putnam Co., in southern N. Y. It may be noted that, although Arthur (**1**) may have been justified in correlating this rust with *Puccinia mariae-wilsoniae*, the correlation is not perfect. The



FIGS. 1–2.

larger and thicker-walled aeciospores and the differently sculptured teliospores indicate that the *Uromyces* is not simply a one-celled variant of the *Puccinia*.

Endophyllum lacus-regis Savile & Parmelee sp. nov.

Pycnia telia aecidioideaque praesertim ephiphylla a mycelio systemico perennique nascentes, fere paginam totam occupantes; plantae infectae steriles saepe foliis multis angustis rigideque erectis (FIG. 2). Telia bullata poro centrali dehiscentes, nunquam cupulata (FIG. 1, supra). Peridium subtile, cellulis laxe junctis et globosis vel compressis ellipsoideis 15–50 × 12–43 μ; paries 2.0–6.0 μ crass. verrucis inclusis, hyalinus, leviter striatus vel irregulariter rugosus verrucis usque ad 3.5 μ alt. Peridium pseudoperidio plectenchymato firmo protectum. Teliosporae aeciosporas simulantes, 22–35(–38) × 18–29(–33) μ, intus saturatim rubro-aurantiacae; paries 1.5–2.5(–3.3) μ crass., hyalinus, subtiliter vel moderate verrucosus verrucis rotundatis vel ± labyrinthiformis; pori germinativi invisi; basidio germinantes vere proximo.

On *Claytonia caroliniana* Michx., moist beech-maple woods, Kingsmere road, between Kingsmere and Old Chelsea, Gatineau Co., Que., 13 May 1955, D. B. O. Savile and J. A. Parmelee (DAOM *46405*, **TYPE**). First found at type locality 27 May 1948 (DAOM *20625*), and found abundantly each year since at this site, where the rusted plants are abundant in about one-half acre and are scattered over about two acres. Isotype distribution: BPI, FH, G, IARI, IMI, K, MICH, O, PUR, S, TRT, UC, UPS, WIS, WS, Tokyo University of Education, Forstlige Forskningsanstalten Helsinki, and extra packets to BPI. Until 1955 no other colony of this rust was found although the host is abundant locally in suitable habitats. In 1955 a great many *Claytonia* stands in Gatineau Co., Que., and adjacent Carleton Co., Ont., were searched. Five individual, widely scattered, infected plants were found in this systematic search, and Dr. F. L. Drayton found a colony about 20 yd. diam. (DAOM *46406, 46407, 46408, 46409, 46410, 46411*). All these stands were within 2 mi. NW, N or NE of Kingsmere, except for a solitary plant about 3 mi. NE; hence the specific epithet. Recently, however, Parmelee and R. A. Shoemaker (DAOM *46412*) have found a colony near Hope Bay, Bruce Co., Ont., some 270 mi. west and slightly south of the earlier finds.

Endophyllum lacus-regis develops later than the aecial stage of

FIGS. 1–2. 1. Leaves of *Claytonia caroliniana* × ca. 5; bullate aecidioid telia of *Endophyllum lacus-regis* above, and cupulate aecia of *Puccinia mariae-wilsoniae* below. 2. *Claytonia carolianiana;* healthy plant on left; plant infected by *Endophyllum lacus-regis* on right, showing proliferation, suppression of flowering, and narrowing of leaves.

Puccinia mariae-wilsoniae, its pycnia being evident when the aecia of the latter are open, and its telia maturing slightly before those of the *Puccinia*. Although, at a casual glance, the telia might pass for the aecia of *P. mariae-wilsoniae*, it is hard to believe that such a conspicuous rust could have escaped observation for so long unless it were scarce or absent over most of the range of the host. Southern Quebec, southern Ontario and the northeastern United States have been so thoroughly botanized that we are inclined to believe that the true distribution of *Endophyllum lacus-regis* may be not unlike that indicated by the specimens, i.e. sparingly along the northern limits of *Claytonia caroliniana*. Many *Claytonia* stands become so overgrown by other herbaceous plants, by the time this rust is mature, that we have found it advisable to search for it as soon as the pycnia develop (during the early part of the flowering season and concurrently with the aecia of *P. mariae-wilsoniae*). Although the pycnia are not easily seen without stooping, one can with practice recognize infected plants by their habit. The groups of narrow, erect leaves often look much like a group of maple cotyledons springing from a squirrel cache. FIG. 2 shows healthy and infected plants from corms of approximately equal size. There is great variation in the number of flower stalks and basal leaves produced from both healthy and infected corms, but the numbers are usually much higher in infected than in healthy plants. Weakly developed raceme initials are sometimes produced by rusted plants, but the individual flower buds seem never to be formed. Usually one finds only a small initial between the pair of cauline leaves. FIG. 1 shows that the sori are more widely spaced than the aecia of *P. mariae-wilsoniae* and also indicates their different appearance.

The biology of *Endophyllum lacus-regis* is not yet known in detail. When we first found the "aecia," we looked for uredinia or telia on *Claytonia* or some other plant. Eventually, when none could be found, repeated attempts were made to germinate the spores, completely without success, and the nature of the organism was then suspected. After further failures, slides with heavy spore deposits were prepared in May 1954. The deposits were covered with 22 × 50 mm cover slips tacked in place with nail lacquer but not completely sealed. These slides were protected with perforated wooden containers and placed in soil and forest litter at various depths in two locations. Despite human interference at both sites a few surviving slides showed complete germination on recovery and examination on 27 April 1955. The empty spores were, in all such slides, accompanied by numerous ellipsoid, yellow-pigmented, thin-walled spores 15–20 × 9.15–15 μ, with no apiculus and no sign of

germ-tubes. There was abundant free moisture on all the slides and it is tentatively assumed that these spores are derived from the fragmenting of basidia, a well-known reaction when basidia cannot rise clear of the enveloping film of water. At least there is no evidence of germ-tube germination, and the assignment to *Endophyllum* accordingly seems to be justified. It is probable that under suitable conditions typical basidiospores are produced on sterigmata, although they may be difficult to demonstrate. The spores observed have consequently been excluded from the diagnosis.

Observation of five completely isolated plants as well as of those scattered round the margins of colonies has shown that such plants regularly mature their telia as promptly as those in the heart of a colony. It thus appears that the rust is self-fertile despite the production of numerous pycnia with copious nectar and apparently normal pycniospores.

Rusts such as *Endophyllum lacus-regis* presumably arise from ancestral long-cycled species that have systemic aecia or telia. No such rust is known on Portulacaceae, and the origin of this species is as puzzling as its apparent distribution.

PUCCINIA CLAYTONICOLA Cumm., Bull. Torrey Bot. Club **79**: 218. 1952.

This recently described rust was originally discovered by Dr. W. G. Solheim in the Medicine Bow Mts., Wyo., on 8 July 1947. J. A. Calder and Savile found it to be abundant on the type host on the lush subalpine meadows of Mt. Revelstoke National Park, B.C., on 22 July 1953. This collection agrees closely with type material supplied by Dr. Cummins. Our extensive material shows that the rust is unquestionably microcyclic and that it is not systemic, although the sori, like the aecia of *Puccinia mariae-wilsoniae*, may be extensive. Our collection yields spores 20–30 × (13–)14.5–19 μ, not or slightly constricted at septum; wall 0.8–1.6 μ thick, light chestnut, smooth except for a few scattered delicate ridges or rows of small warts, usually 2–6 μ apart, ca. 0.3–0.4 μ high and 0.3–0.4 μ wide, running longitudinally or diagonally, or occasionally only a few scattered warts; germ-pores apical to slightly depressed and (¼–)⅔–¾ depressed, with a very small papilla or none; pedicel hyaline, deciduous. In the type collection the spores run to 36 μ long and 20 μ wide, but in all other respects the two collections are indistinguishable. Duplicates of the Mount Revelstoke collection have been distributed to all those institutions with whom we exchange, and a number of extra packets have also been sent to the National Fungus Collections, Beltsville, Md., in view of the limited distribution possible for the type collection.

Claytonia lanceolata was found frequently in southern British Columbia in 1953, in lowland and alpine or subalpine situations, but the rust was not seen elsewhere. The alpine stands are generally lusher than those in the lowlands, and the moist weather of the alpine meadows is probably more suitable for infection than that of spring in the semi-arid lowland valleys. The *Claytonia* stands on Mt. Revelstoke were the most extensive of any seen. That so widespread and conspicuous a rust should have been overlooked for so long reflects our superficial knowledge of the Cordilleran alpine fungus flora.

PUCCINIA CLAYTONIAE Thuem., Bull. Soc. Imp. Nat. Moscou **53**: 217. 1878.

This species, known only from south-central Siberia on *Claytonia ioanneana* (first reported as *C. arctica*, but corrected by Tranzschel, **9**), is mentioned here because it appears from the description recorded by Sydow (**8**) to be closely related to *Puccinia claytoniicola*. It was stated to have teliospores 24–28 × 18–21 μ, not constricted, with pale brown, smooth, very thin wall and "pedicello subnullo" (i.e. deciduous). Thus the main distinction seems to be the presence or absence of warts on the spore walls. The distribution and morphology of these two rusts suggest that they diverged from a common ancestor in interglacial or late preglacial times.

UROMYCES UNITUS Peck, Bull. Torrey Bot. Club **10**: 74. 1883.

Interest in this rust was aroused by the realization that the rust on *Lewisia rediviva* from the Okanagan Valley, B. C., was intermediate in morphology between the rust on *L. rediviva* in Montana and *U. spragueae* on *Spraguea umbellata* in the Sierra Nevada. The latter, in turn, proved to be distinct from the rust on the same host in coastal California. The available specimens of these rusts have been critically examined, sorted according to morphology, and their distribution plotted (FIG. 3). It will be seen that the complex falls into four geographically distinct populations, and that the segregation is to a considerable degree independent of host identity. It accordingly seems most practical to treat these populations as subspecies of *U. unitus* as below. It may be argued that, as these rusts lack pycnia and presumably are obligately self-fertile, they cannot intergrade and are full species. Although such a view may be theoretically correct, its acceptance would involve an excessively narrow species concept, quite at variance with that com-

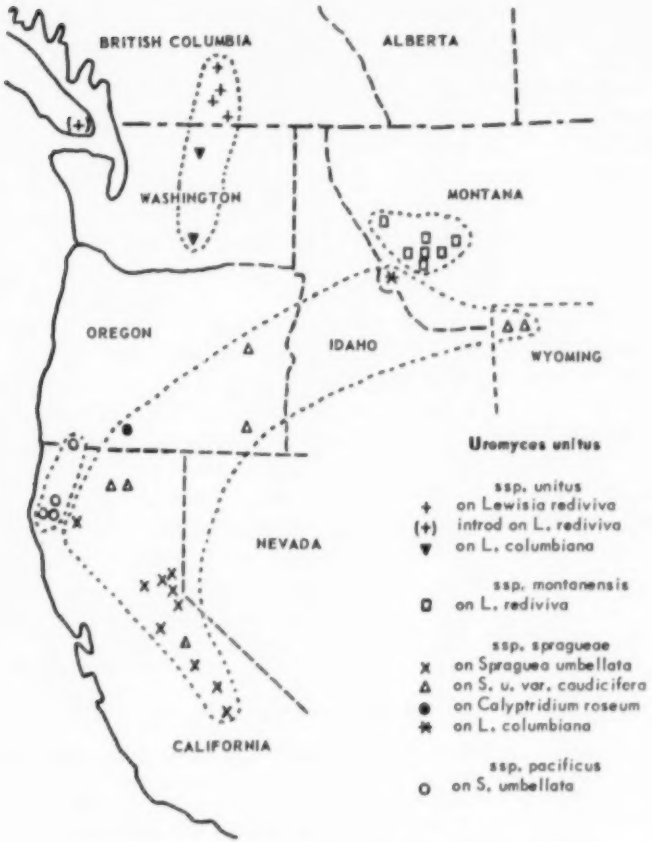


FIG. 3. Distribution of the subspecies of *Uromyces unitus*.

monly employed for the rusts and seriously complicating the construction and use of keys.

UROMYCES UNITUS Peck ssp. **unitus**

Pycnia unknown, apparently lacking. Aecia amphigenous, cupulate. Aeciospores (15–)17.5–22 × (12.5–)14–17.5(–19) μ; wall 0.6–1.0 μ thick, hyaline, minutely verrucose. Telia amphigenous, blackish brown. Teliospores 25–38.5 × 22–31.5 μ; wall 1.5–3.5 μ thick at sides, 4.0–7.5 μ at apex excluding warts, chestnut, shallowly rugose with irregular warts

or ridges 0.1–0.3 μ high at sides, 0.2–0.5 μ high at apex, often ± reticulate with meshes 1.3–2.2 μ diam., sometimes longitudinal on part of spore; pedicel hyaline to yellowish, deciduous to weakly persistent.

On *Lewisia columbiana* (Howell) B. L. Robinson, "Washington Territory," T. S. Brandegee, n.d. (apparently 1882) (PUR ex Peck, **TYPE**); War Creek, near NW end of Chelan L., Wash. (BPI ex WS *956*). On *L. rediviva* Pursh, Summerland, Kaledon, White L. road near Kaledon, and Anarchist Mtn., all southern Okanagan Valley, B. C.; and North Saanich, Vancouver I., B. C., on plants introduced from Okanagan Valley (all in DAOM). As Ewan has indicated (**3** and *in litt.*), Brandegee's Washington specimens have given considerable trouble. *Lewisia columbiana* is alpine or subalpine and a Washington specimen would almost certainly be from the Cascades. A *Heuchera* collected by Brandegee in the Cascade Mts., Wash., in 1882, and recently examined, must have come from the east slope of the Cascades in southern Washington, much farther south than the other cited specimen on *L. columbiana*. Morover, Ewan (**3**) records Brandegee as having been in the Yakima district in 1882. (He was in Washington in 1882 and 1883, but the latter year is inadmissible.) The presumptive site of the type collection has been plotted on this basis. It is possible that the Okanagan Valley collections on *L. rediviva* represent a distinct population, but they are much closer to the Washington specimens on *L. columbiana* than to the Montana ones on *L. rediviva*.

UROMYCES UNITUS Peck ssp. **montanensis** Savile ssp. nov.

Aecia invisa. Teliosporae 26.5–38.5(–40) × 21–31.5(–33.5) μ; paries 2.0–4.0 μ in latere, 4.5–8.0(–9.0) μ in apice verrucis exclusis, castaneus, levis vel minute partim striate partim reticulate rugosus in latere, levis vel rugosus columinibus usque ad 0.3(–0.5) μ alt. in apice; pedicellus hyalinus vel subhyalinus, ± deciduus.

On *Lewisian rediviva* Pursh, Deer Lodge, Mont., F. D. Kelsey, May 1889 (F. Columb. *1373* in DAOM, **TYPE**); Barth. N. A. Ured. *1100*, perhaps a duplicate of the type; three other collections from Deer Lodge by Kelsey (PUR); Missoula, Mont. (PUR); and Helena, Mont. (PUR). All sites are in western Montana.

UROMYCES UNITUS Peck ssp. **spragueae** (Harkn.) Savile n. comb.

Uromyces spragueae Harkn. Bull. Calif. Acad. **1**: 36. 1884.

Pycnia apparently lacking. Aecia amphigenous. Aeciospores (16–) 17.5–23.5 × 14–17.5 μ; wall 0.7–1.0 μ thick, hyaline, minutely verrucose.

Telia dark brown. Teliospores 23–36(–39) × 18.5–28.5 μ; wall 1.5–3.0(–3.3) μ at sides, (2.2–)3.0–6.0 μ at apex excluding warts, light chestnut, conspicuously rugose with irregular, broken, anastomosing or occasionally ± longitudinal ridges 0.1–0.5(–0.9) μ high at sides, 0.2–0.8(–1.2) μ high at apex, sometimes forming a reticulum over part of spore with meshes 1.3–2.4 μ diam., rarely ± smooth toward base of spore; pedicel hyaline to subhyaline, deciduous.

On *Spraguea umbellata* Torr.: Nine collections from Sierra Nevada, Calif., all agree closely, including Rab.-Wint. F. Eur. *3309* (**TYPE**), Rab.-Wint.-Paz. F. Eur. *3933*, Barth. N. A. Ured. *1297*, and six other specimens in PUR. The exact localities for three specimens are uncertain, but two, including the type, are believed to have been near Truckee; the third has simply been plotted in the middle of the range. A single specimen from Humboldt Co., Calif. (UC *471897*) impinges on the range of the next subspecies. On *Spraguea umbellata* Torr. var. *caudicifera* A. Gray: Donahue Pass, Yosemite Nat. Park (PUR *43736*); Wagon Camp, Mt. Shasta (UC *788785*), and Mt. Shasta (Mycob. of N. Am. *18*), all Calif.; Strawberry Mt., Grant Co. (PUR *34977*), and Steens Mts. (PUR *46734*), Ore.; Grand Canyon (PUR *34976*) and Norris Geyser Basin (PUR *34978*), Yellowstone Nat. Park, Wyo. Assigned here with slight reservations are single specimens on *Calyptridium roseum* S. Wats., Crater L., Klamath Co., Ore. (PUR *34964*); and on *Lewisia columbiana* (Howell) B. L. Robinson, Tin Cup Canyon, west of Darby, Mont. (PUR *52832*).

UROMYCES UNITUS Peck ssp. **pacificus** Savile ssp. nov.

Aecia amphigena. Aeciosporae 19–24 × 13–20.5 μ; paries 0.8–1.2 μ crass., hyalinus, subtiliter verrucosus. Telia brunnea, amphigena. Teliosporae 26–36(–39) × 20.5–28 μ; paries (1.0–)1.5–3.2 μ crass. in latere, 3.0–4.5(–5.0) μ crass. in apice verrucis exclusis, brunneus, levis vel minute rugosus verrucis irregularibus vel columninibus sinuosis ca. 0.1 μ alt. in latere, rariter usque ad 0.4 μ alt. in apice, interdum reticulam efficientes; pedicellus hyalinus, deciduus.

On *Spraguea umbellata* Torr., Grouse Mtn., Humboldt Co., Calif., J. P. Tracey *12705* (PUR *46735*, **TYPE**); Grouse Mtn. (PUR *48240*), and Trinity Summit (UC *555013*), Humboldt Co., Calif., J. P. Tracey; east of Oregon Caves, Calif.-Ore. boundary, H. E. Parks (PUR *46737*). This subspecies appears to be quite strictly coastal.

It will be clear from the descriptions that these four taxa are extremely closely related. If they spread through each others' range, as might eventually happen, the differences would probably be ascribed to

chance. The only appreciable distinctions are in teliospore wall thickness and intensity of wall sculpturing. The pattern of sculpturing is essentially the same in all subspecies, being intermediate between striate and reticulate. As has been shown elsewhere (**6**), these two patterns transform into one another with a small change in cytoplasmic instability; and it is remarkable that the intermediate condition has persisted throughout the series.

Such sharply delimited distribution patterns as that of *Uromyces unitus* are not common in the rusts. Obviously the high relief and climatic discontinuities of the Cordillera encourage isolation; but a full explanation of the pattern shown by the subspecies of this rust is difficult to visualize. The broad sweep of ssp. *spragueae* from Oregon across to Wyoming suggests that this rust spread across this region with its host plants at about the glacial maximum when even the lowlands would have been cool enough to support *Spraguea*. The currently almost contiguous ssp. *montanensis* is rather sharply distinct from ssp. *spragueae* in morphology and must have been isolated from it for a considerable time. It perhaps persisted in a refuge on the east slope of the Rockies during a glacial period when the main population was pushed southward. The present distribution of ssp. *unitus* to the east of the crest of the Cascades in Washington and southernmost British Columbia is the pattern for several plants of this region. When a marked drying and warming of the climate initiated the retreat of the last Cordilleran ice sheet, the east slope of the Cascades probably became warm enough to support a substantial flora. Many plants may have spread northward in this area while the ice sheet still persisted to the east. The appreciable distinction between ssp. *unitus* and ssp. *spragueae* indicates a substantial period of geographic or physiological isolation. If the indicated distribution of ssp. *unitus* is approximately complete, there is a strong inference that it persisted through the last glaciation in a refuge in the southern Cascades; for both *Lewisia columbiana* and *L. rediviva* occur southward to California, and if the rust spread into the Cascades only after the retreat of the ice sheet one would expect it to occur in Oregon and California today. A glacial period presumably brought *Spraguea umbellata* or its immediate ancestor into the lowlands between the Sierra Nevada and the Coast Range of California; and ssp. *pacificus* may be supposed to have differentiated during subsequent isolation in a xerothermic period. At some probably quite recent time ssp. *spragueae* has become at least sparingly established in the Coast Range. The latter subspecies has more strongly sculptured, and hence more buoyant, teliospores than the

other members of the complex, and is accordingly the best adapted to extend its range during periods when gaps in host distribution are wide.

KEY TO NORTH AMERICAN RUSTS OF PORTULACACEAE

1. Aecia present, cupulate, often diffuse but not systemic; telia scattered.....2
1. Aecidioid telia present, bullate, systemic.....*Endophyllum lacus-regis*
1. Aecia lacking; telia in circular groups.....8
2. Pycnia present.....3
2. Pycnia lacking; teliospores 1-celled, closely rugose.....5
3. Aeciospores 14–20 μ wide; teliospores 2-celled, uniformly verrucose.....4
3. Aeciospores 22–24.5 μ wide; teliospores 1-celled, sparingly warted .....*Uromyces claytoniae*
4. Teliospores (30–)33–56 × 16.5–27 μ, eastern, on *Claytonia* .....*Puccinia mariae-wilsoniae* var. *m.-w.*
4. Teliospores 27–46(–48) × 20–28.5 μ, western, on *Montia* .....*P. mariae-wilsoniae* var. *montiae*
5. Teliospore wall 4–8 μ at apex excluding warts.....6
5. Teliospore wall 3–6 μ at apex excluding warts.....7
6. Teliospores definitely rugose on sides, ridges to 0.5 μ high at apex .....*Uromyces unitus* ssp. *unitus*
6. Teliospores often smooth on sides, ridges rarely over 0.3 μ high at apex.....*U. unitus* ssp. *montanensis*
7. Teliospores conspicuously rugose on sides, ridges 0.2–0.8(–1.2) μ high at apex.....*U. unitus* ssp. *spragueae*
7. Teliospores smooth to very shallowly rugose on sides, ridges rarely to 0.4 μ high at apex.....*U. unitus* ssp. *pacificus*
8. Pycnia usually evident; teliospores 30–48 × 19–30 μ, uniformly verrucose.....*Puccinia agnita*
8. Pycnia lacking; teliospores 20–36 × 14–20 μ, with scattered ridges of rows of warts.....*P. claytoniicola*

USTILAGINALES

Ustilago nelsoniana Savile sp. nov.

Teliosporae 10–16.5 × 9–13 μ; paries ca. 0.3–0.5 μ crass., brunneo-purpureus, reticulatus scutulis irregularibus 0.2–1.2 μ alt., 0.5–2.2 μ lat. vel imperfectibus.

In anthers of *Montia cordifolia* (S. Wats.) Pax. & K. Hoffm., outlet of Cottonwood L., 4 mi. SSE of Nelson, B. C., 2900 ft., 19 June 1953, J. A. Calder and D. B. O. Savile *9380* (DAOM *44131*, **TYPE**). Duplicates distributed to BPI, FH, IARI, IMI, TRT, UC, UPS, WS.

The only recorded anther smut of Portulacaceae is *Ustilago calandriniicola* Speg. (Anal. Mus. Nac. Buenos Aires **19**: *287*. 1909) on *Calandrinia affinis* in Argentina. According to Zundel (**11**) this species has spores 5–7(–10) μ diam., and it is clearly distinct. Although *U.*

nelsoniana is the only anther smut reported on North American Portulacaceae, two ovariicolous species have been described. Dr. Geo. W. Fischer has supplied slides from the type collections of these two smuts, from which the following descriptions have been prepared.

USTILAGO CALANDRINIAE G. P. Clint., Proc. Bost. Soc. Nat. Hist. **31**: 378. 1904.

Teliospores 16.5–19 × 13.5–17.5 μ; wall ca. 0.4 μ thick, purple-brown, often with thicker, subhyaline inner layer, conspicuously reticulate with meshes 1.5–3.3 μ high, 0.8–2.0 μ wide. On *Calandrinia breweri* S. Wats., Santa Barbara, Calif.

USTILAGO CLAYTONIAE Shear, Bull. Torrey Bot. Club **34**: 317. 1907.

Spores 13.5–18.5 × 12.5–17.5 μ; wall ca. 0.5 μ thick, light purple-brown, conspicuously reticulate with meshes 1.0–2.8 μ high, 1.3–3.0 μ diam. at base or occasionally to 3.5 μ long. On *Montia linearis* (Dougl.) Greene, Vancouver Barracks, Wash.

These two smuts are quite distinct from *Ustilago nelsoniana*, but very closely related to each other. They appear to be validly distinguishable by their spore-wall reticulations.

Nelson, B. C., is almost the northern limit of *Montia cordifolia*. *Ustilago nelsoniana* should accordingly be sought in the Pend Oreille River country to the south.

The four purple-spored species of *Ustilago* attacking Portulacaceae may be separated thus:

1. Teliospores 5–7(–10) μ diam. ..... *U. calandriicola*
1. Teliospores 10–16 × 9–13 μ ..... *U. nelsoniana*
1. Teliospores 14–19 × 13–17 μ 2
   2. Spore-wall meshes 1.5–3.3 μ high, 0.8–2.0 μ diam. ..... *U. calandriniae*
   2. Spore-wall meshes 1.0–2.8 μ high, 1.3–3.0(–3.5) μ diam. ..... *U. claytoniae*

PHYCOMYCETES

PERONOSPORA CLAYTONIAE Farl., Bot. Gaz. **8**: 314. 1883.

What appears to be this species was found on *Montia perfoliata* (Donn) Howell twice in extreme southern British Columbia in 1953: near Cascade, 22 June, Calder and Savile *9549;* and 8 mi. SW of Okanagan Falls on Orofino Mtn., 1 July, Calder and Savile *9934*. These collections constitute a slight northward range extension, the species being recorded from Washington. A composite description from these specimens is:

Sporangiophores 250–650 μ high, 10–12 μ wide above the very slightly swollen base, widely branched up to 12 times in upper $\frac{1}{4}$–$\frac{1}{3}$, ultimate tips 3.5–11 μ long, straight to slightly curved. Sporangia 20–26 × 14.5–18(–20) μ, pale yellow-brown to violet-brown. Oogonia 42–55 × 36–50 μ diam., wall pale yellow. Oospores 34–39 × 33–38 μ diam., wall dark chestnut.

Except that the oospores are a trifle small these figures match very closely Farlow's for the fungus on *Claytonia virginica* from Kentucky. F. Columb. *3441* on *C. virginica*, London, Ont., is presumably the same, but the fragment available is overmature and the sporangia have collapsed.

PHYSODERMA CLAYTONIANA H. C. Greene, Farlowia **1**: 569. 1944.

This species, so long overlooked, was collected at Laprairie, near Montreal, Que., in 1951, on *Claytonia virginica* by L. Cinq-Mars (DAOM *36466*); and in 1955 on *C. caroliniana* at Lac Philippe (DAOM *46358*) and Yipe Trail, Camp Fortune (DAOM *46433*), both Gatineau Co., Que., by Savile and Parmelee. These specimens agree very closely with the type from Sauk Co., Wis. A composite description from these four collections is:

Resting sporangia 16–25.5(–28) × (12.5–)14–24 μ; wall 0.8–2.0 μ thick, yellow-brown, smooth.

In Essex Co., Ont., and Washtenaw Co., Mich., there is a consistently larger-spored *Physoderma*, which may be either a distinct species or a well-marked local variant. This is the organism which Sparrow (**7**) studied, understandably believing that it was typical *P. claytoniana* and that Greene's description was based on immature material. Actually Greene's specimen is fully mature, as are the others listed above. For the present it seems advisable to treat the large-spored organism as a variety, for which the following name is proposed.

PHYSODERMA CLAYTONIANA H. C. Greene var. **sparrowii** Savile and Parmelee var. nov.

A typo differt sporangiis quiescentibus plerumque 23–34 × 20–29.5 μ, pariete 1.5–2.0 μ crass.

In leaves, stems and inflorescences of *Claytonia virginica* L., near Malden Centre, Essex Co., Ont., 26 May 1950, J. A. Parmelee and I. L. Conners (DAOM *24072*, **TYPE**). This is unquestionably the organism collected by Sparrow (**7**) at Amherstburg, a few miles dis-

tant, and later in Washtenaw Co., Mich., and studied and illustrated by him in detail. It should be noted that the description by Karling (**4**) of *P. claytoniana* is based primarily on this variety.

SYNCHYTRIUM sp.

I. L. Conners and J. A. Parmelee, while collecting *Puccinia mariae-wilsoniae* and *Physoderma claytoniana* var. *sparrowii* near Malden Centre, Essex Co., Ont., on 26 May 1950, found a *Synchytrium* on *Claytonia virginica* L. Although he is unwilling to name it without having been able to germinate the spores, Dr. John S. Karling has kindly provided a description of this organism to add to the completeness of this report.

"Resting sporangia usually solitary, occasionally 2 in a cell, predominantly subspherical to spherical, 56–80 μ, or oval, 48–60 × 58–72 μ, with a smooth dark brown, 3–4.2 μ thick wall and coarsely granular content; enveloped by a crust of crumbly residue which may fill remainder of host cell; germination unknown.

"Compositely monogallic, resting spore galls usually separate and scattered, or aggregated and occasionally confluent, protruding fairly conspicuously, brownish-black at maturity, almost hemispherical, 108–170 μ diam., with a sheath 2–4 cells thick."

A portion of the collection (DAOM *45856*) on which this description is based was sent to the late Dr. M. T. Cook, and Dr. Karling has another fragment. Another collection made slightly farther west and mixed with *Physoderma claytoniana* var. *sparrowii* was sent to Dr. F. K. Sparrow. A recent search of phanerogamic sheets in DAO has yielded a further specimen (DAOM *46434*) of presumably the same species on *Claytonia virginica* at St. Jean, Que., about 25 mi. SE of Montreal, coll. M. Raymond and J. Kucyniak. This inconspicuous parasite is presumably much commoner than the records suggest.

Although these specimens might be placed in *Synchytrium aureum*, there can be no certainty as to subgeneric assignment until the resting spores can be germinated.

ALBUGO PORTULACAE (DC.) O. Kuntze, Rev. Gen. Plant. **2**: 658. 1891.

This common parasite of *Portulaca oleracea* L. is represented in DAOM from many localities in Ontario and Quebec and sparingly from Manitoba and Saskatchewan. It is also reported from New Brunswick and Nova Scotia by Wehmeyer (**10**). It is sometimes very locally

destructive under favorable conditions, but is probably not important in controlling this weed.

FUNGI IMPERFECTI

HELMINTHOSPORIUM PORTULACAE Rader, Mycologia **40**: 344. 1948.

Helminthosporium portulacae was described from Watkins Glen, N. Y., on *Portulaca oleracea* L. J. Sibalis and I. L. Conners (in Conners and Savile, 2) report it on *P. grandiflora* Hook. from Gananoque, Ont., where it was suspected to have occurred first in 1940, and on *P. grandiflora* and *P. oleracea* from Macdonald College, Que. E. T. Reeder, in the same report, records it on *P. oleracea* from Saskatoon, Sask.

SUMMARY

Detailed descriptions are given of the following rusts of *Claytonia: Puccinia mariae-wilsoniae, P. agnita, P. claytoniicola, Uromyces claytoniae* and *Endophyllum lacus-regis* Savile & Parmelee. *P. mariae-wilsoniae* var. *montiae* Savile on *Montia* in the Cordillera is distinguished from the eastern rust on *Claytonia*. It is shown that the *Uromyces unitus* and *U. spragueae* complex is best treated as four subspecies: *U. unitus* ssp. *unitus* in B. C. and Wash., ssp. *montanensis* Savile in Montana, ssp. *pacificus* Savile in NW coastal Calif., and *spragueae* (Harkn.) Savile in the Sierra Nevada and from SE Ore. to NW Wyo. *Ustilago nelsoniana* Savile is described from B. C. on *Montia cordifolia* and compared with *U. calandriniae, U. calandriniicola,* and *U. claytoniae*. *Physoderma claytoniana* is reported on *Claytonia* spp. from Que., and var. *sparrowii* Savile & Parmelee is described from Essex Co., Ont. *Synchytrium* sp. is recorded on *Claytonia virginica* from Ontario and Quebec. Other parasites are mentioned.

ACKNOWLEDGMENTS

We are indebted to Dr. Geo. B. Cummins (and in his absence, Dr. Forest W. Stearns), Dr. Geo. W. Fischer, Dr. H. C. Greene, Mr. Stanley J. Smith, and Mr. John A. Stevenson for the loan of specimens; to Dr. Lee Bonar for specimens and information on the positions of several California specimen sites; to Dr. Joseph Ewan for information on T. S. Brandegee's collections; to Dr. John S. Karling for advice on the disposition of *Synchytrium* and *Physoderma* specimens; and to Dr. Robert E. Woodson, Jr., for information on Trelease's Alaskan collections. Dr. Cummins also revisited Tin Cup Canyon, Mont., in 1955 and

confirmed that the rust previously collected there on overmature plants was actually on *Lewisia columbiana*.

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NOMENCLATURAL NOTES. I. MISUSE OF NEOTYPES FOR VENTURIA AND PHAEOSPHAERELLA

RICHARD P. KORF [1]

> "A *neotype* is a specimen selected to serve as nomenclatural type for so long as all of the material on which the name of the taxon was based is missing." (International Code of Botanical Nomenclature, 1952, Art. 18, Note 3.)

A scientific worker who attempts to follow the International Code and wishes to place a valid name on a specimen of the common apple scab organism or one of its allies is rapidly made aware that he is entangled in the meshes of a thoroughly confusing nomenclatural (and even taxonomic) problem. He can find good authority for placing the fungus involved in any one of three genera: *Venturia, Endostigme,* or *Spilosticta.* If he is truly persistent, he will find that the valid generic name for his organism is *Phaeosphaerella,* which no authority seems to use!

Venturia was first published by De Notaris (**1844, 1845**) to include only two species, *V. rosae* De Not. and *V. dianthi* De Not. One of these two species must serve as the lectotype of the genus. Cesati and De Notaris (**1863**) later added several species to the genus, and retained both of the original species. In the Sylloge Fungorum, Saccardo (**1882**) inexplicably credited the generic name to "De Notaris and Cesati."

Apparently the first designation of a lectotype for *Venturia* from between *the only two species eligible* for consideration was made by Theissen and Sydow (**1917**), who pointed out that, since *V. dianthi* does not fit the generic description, has muriform spores and belongs elsewhere, *V. rosae* must be considered the [lecto]type.

Since *V. rosae* must be the lectotype of *Venturia* De Not., its identity should determine the application of this generic name. However, it appears to have remained a "lost" species for many years. In an effort to settle this point, I have been in correspondence with Dr. L. E. Wehmeyer,

[1] I wish to express my appreciation to the many colleagues and students without whose help and criticism the development of the concepts expressed in this and succeeding papers would never have been possible.

Dr. J. A. von Arx, and Dr. E. Müller. Dr. Müller has fortunately collected this species twice in 1955, and both he and von Arx are of the opinion (*personal communications*) that this species, with its superficial ascocarps developing upon a hypostroma, is best referred to the genus *Gibbera* Fries (**1849**). Since *Venturia* De Not. predates *Gibbera* Fr., it would have to replace that generic name if the International Code is applied. Conservation seems the only solution to avoid the many name changes which would be involved; Dr. Müller advises me that he and a co-author are proposing the new combination for *Venturia rosae* in the genus *Gibbera*.[2]

Saccardo (**1882**), Shear (**1948**), Müller and von Arx (**1950**), von Arx (**1952**), Hughes (**1953**) and all other authors who today use the generic name *Venturia* for the apple scab organism and its allies are guilty of utilizing a neotype (*V. inaequalis*, usually) applied in opposition to the Code. In nearly all recent treatments both of the original species of *Venturia* have been excluded from the genus, whereas the Code demands that one of the two original species must be accepted as the lectotype.

In a paper by Berlese (**1887**), the genus *Protoventuria* Berl. & Sacc. is erected with *V. rosae* De Not. as its holotype. Since *V. dianthi* had been shown to be a *Pyrenophora* (or *Pleospora* as confirmed by Dr. Wehmeyer in a personal communication), and since Saccardo had emended *Venturia* to exclude both *V. dianthi* and *V. rosae*, the new genus was established to accommodate the latter species. This solution is in flagrant violation of the present Code, and the name *Protoventuria* must be rejected as a superfluous name. The correct solution, under our Code, would have been to retain *Venturia* with *V. rosae* as its type and to erect a new genus for the "emended" genus *Venturia* sensu Saccardo.

Phaeosphaerella was erected by Karsten (**1888**) to include a single species, *P. maculosa* (Sacc.) Karst., which is thus the holotype of the genus. Later authors have recognized the identity of this species with *Sphaeria macularis* Fries; more important, however, the species has been recognized as being congeneric with the apple scab organism, with the resultant synonymy: *P. maculosa* (Sacc.) Karst. = *P. macularis* (Fr.) Trav. = *Spilosticta macularis* (Fr.) Petrak = *Venturia macularis* (Fr.) Müller & von Arx.

When Petrak (**1940**: 250) treated the genus *Phaeosphaerella*, he excluded its holotype, in opposition to the Code, on the basis that the

[2] Müller, E. & R. Menon. 1955. Über *Venturia rosae* de Not. und die Nomenklatur der Gattung *Venturia* de Not. Phytopath. Zeitschr. **25**: 190–195.

holotype does not agree with the generic description, and placed that species in *Spilosticta*. He retained the name *Phaeosphaerella*, however, designating *P. typhae*[3] as a neotype, even though the holotype was known. *P. typhae* was said by Petrak to agree more closely with the generic description; such action is, of course, contrary to the type method and cannot be sanctioned under the Code.

Sydow (**1923**) recognized that using *Venturia* for the apple scab organism and its allies was invalid. For this group of important plant pathogens he proceeded to erect two new genera, *Spilosticta* for those with an *Ovularia* imperfect state and *Endostigme* for those with a *Fusicladium* state (or, as we now know from the work of Hughes (**1953**), with a *Fusicladium* or a *Spilocaea* state). On taxonomic grounds, Petrak (**1924**, **1940**) synonymized these genera, choosing *Spilosticta* as the name for the combined genus.

Under the present Code, it is impossible to continue our use of *Venturia* for the important genus of plant pathogens that includes the apple scab organism, and instead the name must apply to a wholly different group of fungi now known as *Gibbera*. The resultant synonymy and typification is:

Venturia De Not. (**1844**, **1845**)
Syn.: *Gibbera* Fr. (**1849**) fide Müller (*in press*)
Protoventuria Berl. & Sacc. *in* Berl. (**1887**)
Lectotype: *V. rosae* De Not., effectively designated by Theissen & Sydow (**1917**: 394).
Spp. alt.: "*G.*" *vaccinii*, etc., etc.[4]

Phaeosphaerella Karst. (**1888**)
Syn.: *Venturia* Auct. non De Not.
Endostigme Syd. (**1923**)
Spilosticta Syd. (**1923**) emend. Petrak (**1924**)
Holotype: *P. maculosa* (Sacc.) Karst. = *P. macularis* (Fr.) Trav.
Spp. alt.: "*V.*" *inaequalis*, "*V.*" *pirina*, etc., etc.[4]

[3] There is an involved nomenclatural problem in deciding how to cite the authorities of this species. The reader is invited to attempt a solution and is referred to Petrak's paper (**1940**). Dr. von Arx has suggested (*pers. comm.*) that it would be appropriate to note here that *Phaeosphaerella* sensu Petrak = *Monascostroma* v. Höhn.

[4] I naturally do not intend to offer new combinations in either *Venturia* or *Phaeosphaerella* because of my proposal for conservation of *Venturia* Sacc. over *Venturia* De Not. If this proposal is not approved by forthcoming International Congresses, however, the new combinations will become necessary.

A PROPOSAL FOR THE CONSERVATION OF VENTURIA AS CURRENTLY USED

If we are to avoid the inconvenience and wholesale name-changing that would result from an application of the Code to the genus of fungi that includes the apple scab organism, it will be necessary to conserve the name for this group of fungi. The following proposal is thereby made to the Committee for Fungi and Lichens of the International Botanical Congress, in keeping with the suggestions of Sprague and Fosberg (**1951**).

NOMEN CONSERVANDUM PROPOSITUM: *Venturia* Sacc. (as *Venturia* De Notaris & Cesati), Syll. Fung. **1**: 586. 1882.

Typus: *V. inaequalis* (Cooke) Wint. *in* de Thüm.

NOMEN REJICIENDUM PROPOSITUM: *Venturia* De Notaris, Giorn. Bot. Ital. **1**(1): 332. 1844; Atti Congr. Sci. Ital. Milano **6**: 484. 1845.

Typus: *V. rosae* De Not.

Reasons for conservation: *Venturia* Sacc. is a large genus of economically important plant pathogens of world-wide distribution (112 spp. in Sacc. Syll.; over 50 spp. fide Engler & Prantl, Natürl. Pflanzenf.; "includes most species in the family [Venturiaceae]" fide von Arx (**1952**), etc.). It is the name used almost universally by plant pathologists, and the name is also used in the large and growing literature relating to the genetics of at least two species. I know of no important flora or monograph of the pyrenomycetes that uses any other name than *Venturia* for these species. If conservation is not effected, there may well be very undesirable consequences, as the name *Venturia* De Not. will compete with *Gibbera* Fr., a large and well-established genus. Failure to conserve would result in lasting confusion and chaos among mycologists, plant pathologists, and geneticists.

Reasons against conservation: The species of the genus of the apple scab organism and its allies have an available, even if unused, generic name (*Phaeosphaerella*). Retention of *Venturia* De Not. would follow the principle of priority. While it is true that the name would thus be used in a manner which has not seen acceptance in the last century, the few authors who have used *Endostigme* and *Spilosticta* appear to approve this course of action (as do Theissen & Sydow, **1917**).

RESULTS OF FAVORABLE ACTION ON CONSERVATION

If the above proposal is acted upon favorably by a subsequent International Congress, the following would be the resultant synonymy and typification (compare above):

Gibbera Fr. (**1849**) emend. Petrak (**1947**) or emend. von Arx (**1952**)

Syn.: *Venturia* De Not. (**1844, 1845**) *nom. rejic.*

Protoventuria Berl. & Sacc. *in* Berl. (**1887**)

Lectotype: *G. vaccinii* (Sow. ex Fr.) Fr.

Venturia Sacc. (**1882**) *nom. conserv.*

Syn.[5]: *Phaeosphaerella* Karst. (**1888**)

Endostigme Syd. (**1923**)

Spilosticta Syd. (**1923**) emend. Petrak (**1924**)

Lectotype: *V. inaequalis* (Cooke) Wint. *in* de Thüm.

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[5] *Sphaerellopsis* Kleb. is also listed as a synonym by von Arx (**1952**), but is only doubtfully validly published, and is also a later homonym of *Sphaerellopsis* Cooke.

PERPETUATION OF THE BROWN RUST OF BARLEY IN ATTICA [1]

P. Critopoulos

Puccinia anomala Rostr. (syn. *Puccinia hordei* Otth.; *Puccinia simplex* Eriks. & Henn.), causing brown rust of barley, is known to have as an alternate host any one of the following 4 species, *Ornithogalum umbellatum* L., *O. pyramidale* L. (syn. *O. narbonense* (L.) A. & G.), *O. tenuifolium* Guss. and *O. pyrenaicum* L.

The life cycle of the fungus was demonstrated for the first time by Tranzschel (**1914**), who used aeciospores from *O. umbellatum*. The work of Tranzschel has been confirmed in the United States by Mains and Jackson (**1924**), who reported the production of the aecial stage as a result of cultures. The connection between *P. anomala* and an alternate host has been confirmed in Austria by Beck (**1924**), in France by Documet (**1926**), in Switzerland by Mayor (**1929**) and in Britain by Dennis and Sandwith (**1948**). The last-named workers inoculated barley plants with aeciospores produced in nature on *O. pyrenaicum* and obtained uredia of *P. anomala* on the inoculated leaves.

In Attica, Greece, the first uredia of *P. anomala* on cultivated barley can be found annually in the beginning of April. On February 20, 1950, however, an area about 1 meter in diameter in which the plants were heavily infected with brown rust was found in a barley planting near Nea Philadelphia, Athens. In other parts of the same planting and in other barley plantings no trace of the disease could be detected at that time. This early and heavy infection of barley coincided with the period of the year (November–April), in which *Ornithogalum* spp. are in leaf in Attica.

Since 1950 several collections of aeciospores from young *Ornithogalum*-like plants, not identifiable because of their early stage of development, were used to inoculate barley plants. One collection made in January, 1954, gave positive results. These were growing in Menidi in a fallow field that had carried barley during the season of 1952–53.

[1] Published by permission of the Director of the Department of Botany, University of Athens, Greece.

MATERIALS AND METHODS

Barley plants of the variety Athinais growing in 20 cm pots filled with ordinary soil were inoculated at stages of growth varying from the seedling stage up to the stage at which the plants were about 30 cm high. The plants were sprayed with a 0.1% agar solution and inoculated by transferring with a needle aeciospores from aecia directly to the barley leaves. Inoculations were made in January and February. The inoculated plants were protected with glass cases and infection was allowed to develop out-of-doors. Control plants were included in all tests.

After performing the inoculations with the various aeciospores that were tested, the aecia-bearing plants were planted in 20 cm pots filled with ordinary soil and were retained until the outcome of the inoculation. The ones that gave the positive result were retained until flowering. At the end of March, 1955, 6 of these plants started to flower but the scape of 4 of them dried when it reached a height of 3–4 cm. The other 2 plants produced a scape 5–8 cm high, which bore 4–5 normal flowers. Two or 3 flower buds at the tip of these scapes dried rather early. One of the flowering plants was left to mature seeds. The ovaries at first developed and then dried out. No seeds were produced.

The alternate host produced numerous bulbils, which were quite uniform in size with a length of 6 mm and a width of 5 mm. A 1-year-old bulbil occasionally formed a single cylindrical leaf. A 2-year-old bulb produced 2 leaves, one of which was more or less cylindrical and the other was channeled, and many bulbils. A 3-year-old bulb produced 4–5 channeled leaves, whose maximum width was 4 mm, a scape, and about one hundred bulbils. The alternate host was identified to be *Ornithogalum umbellatum* L. This species occurs in cultivated areas and stony ground in lower altitudes up to the subalpine region in most parts of the country, including the islands.

The testing of teliospores for germination was made by the usual hanging drop method.

RESULTS

On all inoculated leaves, including the first leaf of seedlings, uredia of *P. anomala* developed in about 10 days. These could be found scattered on both surfaces of the leaves. They were minute, oblong, up to 0.8 mm in length; on the sheaths they were somewhat more elongated. The uredia were erumpent and light orange-brown. On the barley variety used, they were surrounded by chlorotic areas, which often fused to form larger chlorotic spots. In February, telia were not produced on

heavily infected and almost withered leaves. The urediospores were broadly ellipsoid, 19–24 × 21–34 μ, wall straw-colored, 1.5–2 μ thick, finely echinulate, and provided with numerous, scattered pores.

On the infection spots, telia were formed in the middle of April. These were amphigenous, blackish-brown, oblong, often confluent and remained covered by the epidermis for a long time. The sori were divided into compartments by clusters of brown paraphyses, which were flattened at the tip. On leaf blades the telia reached a length of 3 mm whereas on sheaths they reached a length of 6.5 mm. The teliospores were angularly oblong or clavate, 17–22 × 34–56 μ, truncate or obtuse above, narrowed below, slightly constricted at the septum; the spore wall was chestnut-brown and smooth, about 1.5 μ thick at the sides, darker and usually broader near the apex; the pedicel was short and brownish. Mesospores were abundant, 15–28 × 26–41 μ, variable and slightly thickened at the apex (4–6 μ).

THE NATURAL INFECTION OF THE ALTERNATE HOST

On infection spots of the alternate host, groups of pycnia were surrounded by more or less regular circles of aecia. Further out from one of these aecial circles, more pycnia and aecia were produced in varying patterns. Both pycnia and aecia were amphigenous. The pycnia were spherical to ellipsoid, 86–144 × 99–158 μ and were formed below stomata; pycniospores pear-shaped to elongated, 2 × 3–5 μ.

The aecia were yellow and were provided with a colorless peridium. They were elongate-cylindrical, straight or curved, attaining approximately a length of 0.3 mm. In cross section they were round or slightly oblong, about 200 μ in diameter. Young aecia were pointed; upon rupturing, the peridium split irregularly at the tip into recurving segments. The aeciospores were globoid or ellipsoid, 18–23 × 21–33 μ, wall colorless, 1.5–2 μ thick, minutely and thickly verrucose.

As the infected leaf-tips of the alternate host died because of the infection, new pycnia and aecia were produced continuously toward the lower portion of these leaves. At the end of April, when telia had already been produced on the cereal host, the production of new pycnia and aecia was continued.

COMPLETION OF THE LIFE CYCLE

After the production of uredia and telia on barley through the use of aeciospores, a search was made for viable teliospores. In the fall of 1954 teliospores were taken from barley leaves lying on a bare field,

which had carried barley during the season of 1953–54. This field was located adjacent to the field where the infected plants of *O. umbellatum* were collected. The teliospores germinated profusely, producing basidiospores on November 3, 1954, after the long summer, which in this climate is the most adverse period of the year.

In the summer of 1955 temperature measurements of the field, where the leaves with telia were collected, were taken by placing the thermometer on the surface of the bare ground. The temperature at 2 P.M. on July 22 was 45° C, on August 5, was 40° C. These temperatures could be taken as indicative of the temperatures under which the germinating teliospores had survived. Low temperatures, up to the date the spores were tested for germination, rarely if ever go below 10° C, according to data from the Meteorological Institute, Athens.

The life cycle of the fungus is completed by the transportation of the basidiospores to the nearby growing plants of *O. umbellatum*. Ample production of aeciospores then follows, affording inoculum for the barley, which sprouts here at about the middle of October. The uredo stage of the fungus on other gramineous hosts during late summer or fall has not so far been reported for Attica and may not occur.

On plants of *O. umbellatum* growing in the same field where the pycnial and aecial stages of *P. anomala* were collected, the pycnia and telia of *Puccinia Lojkaiana* Thuem. were also commonly found.

SUMMARY

Aeciospores produced in nature on *Ornithogalum umbellatum* L. were used to inoculate barley plants growing in pots. In about 10 days after inoculation uredia of *Puccinia anomala* Rostr. were produced, and these were followed in the middle of April by telia. The pycnia and aecia with their respective spores are described, as are the urediospores and teliospores produced as a result of the inoculations. Teliospores of the pathogen produced during one season and remaining on host parts on the ground under natural conditions were found to produce basidiospores the following November. The life cycle of the fungus is completed by the transportation of the basidiospores to the nearby growing plants of the alternate host. As barley plantings at that time are well advanced, the barley plants become infected and the pathogen is thus perpetuated.

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NOMENCLATURAL CHANGES FOR SOME NORTH AMERICAN UREDINALES[1]

GEORGE B. CUMMINS

The following nomenclatural changes are necessary to bring the names of certain species of North American rust fungi into accordance with the International Code.[2] Most of the changes involve either: 1, the provision of Latin descriptions for telial stages which were previously, but subsequent to January 1, 1935, published in English where a specific epithet based on an imperfect stage was treated as a transfer (technically *nomina nuda*) or 2, names used under a perfect genus but based on the aecial or uredial stage (anticipatory names). In the first case Latin descriptions are necessary to validate the names and in the second case transfer of the specific epithet to the appropriate form genus is required. Names in common usage have been retained wherever possible.

Achrotelium lucumae Cumm. sp. nov.

Uredo lucumae Arth. & J. R. Johnston in Mem. Torrey Bot. Club **17**: 169. 1918; *Uraecium lucumae* (Arth. & J. R. Johnston) Arth. in Bull. Torrey Bot. Club **60**: 467. 1933; *Achrotelium lucumae* (Arth. & J. R. Johnston) Cumm. in Bull. Torrey Bot. Club **67**: 70. 1940 *nom. nud.*

Teliis hypophyllis rotundatis vel ovatis, 0.1–1.0 mm diam. flavidis; teliosporis primo unicellularibus dein ob germinationem 4-cellularibus, ex cellulis basalibus natis, cylindraceis, 15–20 × 59–98 μ, membrana hyalina, 2.5–3 μ cr.; pedicello hyalino, quam spora breviore, persistenti.

TYPE: On *Lucuma nervosa* A. DC., Homestead, Dade Co., Florida, Feb. 7, 1939, Geo. Ruehle.

Bubakia erythroxylonis Cumm. sp. nov.

Uredo erythroxylonis Graz. in Bull. Soc. Mycol. Fr. **7**: 152. 1891; *Bubakia erythroxylonis* (Graz.) Cumm. in Bull. Torrey Bot. Club **67**: 69. 1940 *nom. nud.*

[1] Cooperative investigations between the Purdue University Agricultural Experiment Station and the Plant Disease Epidemics and Identification Section, Agricultural Research Service, United States Department of Agriculture. Journal Paper Number 928 of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology. The assistance of Mr. John A. Stevenson and Dr. D. P. Rogers with some of the nomenclatural problems is gratefully acknowledged.

[2] Lanjouw, J. 1952. International Code of Botanical Nomenclature. 228 pp. Utrecht.

Teliis hypophyllis, atris, circum urediis aggregatis, subepidermalibus, 35–100 μ diam., ex sporis 2–4 irregulariter superpositis compositis; teliosporis variabilis, oblongis vel cuboideis, 7–12 × 12–26 μ, membrana castaneo-brunnea 1 μ cr., ad apicem usque ad 2.5 μ cr.

TYPE: On *Erythroxylon havanense* Jacq., Vivijagua, Isle of Pines, Cuba, Feb. 28–29, 1916, N. L. Britton, E. G. Britton and P. Wilson *15023*.

Chaconia ingae (Syd.) Cumm. comb. nov.

Bitzea ingae (Syd.) Mains in Mycologia **31**: 38. 1939.

In 1949, Thirumalachar and Cummins (Mycologia **41**: 525) reduced *Bitzea* to synonymy with *Chaconia* but the species was not formally transferred.

Chrysomyxa empetri Schroet. sp. nov.

Uredo empetri Pers. ex DC. in Fl. France **6**: 87. 1815; *Thecopsora empetri* (Pers.) Karst. in Bidr. Finlands Nat. Folk **31**: 143. 1879, based on uredia; *Chrysomyxa empetri* (Pers.) Schroet. in Cohn in Krypt. Fl. Schles. **3**(1): 372. 1887, based on uredia; *Chrysomyxa empetri* (Pers.) Rostr. in Medd. om Groenl. **3**: 536. 1888 ***nom. nud.***; *Melampsoropsis empetri* (Pers.) Arth. in Résult. Sci. Congr. Bot. Vienne p. 338. 1906, based on uredia.

Teliis epiphyllis, pulvinatis, flavidis, elongatis, 0.5–3.0 mm longis; teliosporis 3–6 superpositis, plerumque oblongis, 15–24 × 18–45 μ, membrana hyalina, 1 μ cr.

TYPE: On *Empetrum nigrum* L., Kongsvoll in Opdal, Norway, I. Jorstad. (Type in Botanical Museum, Oslo.)

Telia were first described by Jørstad (Kgl. Norske Vidensk. Selsk. Skr. **38**: 51. 1835, issued 1936) followed by Faull (Jour. Arnold Arb. **18**: 147. 1937) but neither description was in Latin.

Chrysomyxa piperiana Sacc. & Trott. sp. nov.

Melampsoropsis piperiana Arth. in N. Am. Flora **7**: 120. 1907, based on uredia; *Caeoma piperianum* (Arth.) Sacc. & Trott. in Saccardo in Syll. Fung. **21**: 787. 1812, based on uredia; *Chrysomyxa piperiana* (Arth.) Sacc. & Trott. in Saccardo in Syll. Fung. **21**: 716. 1912, based on uredia; *Peridermium parksianum* Faull in Jour. Arnold Arb. **15**: 87. 1934.

Teliis hypophyllis, flavidis, pulvinatis, 0.2–0.5 mm diam.; teliosporis 3–6 superpositis, oblongis vel cuboideis, (9–)11–14(–16) × (13–)16–30(–35) μ, membrana hyalina, 1 μ cr.

TYPE: On *Rhododendron macrophyllum* Don (*R. californicum* Hook.), Trinidad, Calif., June 1935, H. E. Parks *5565*.

Telia were first reported by Faull (Jour. Arnold Arb. **17**: 111. 1936) but without a validating Latin description.

Coleosporium eupatorii Arth. sp. nov.

Coleosporium eupatorii Arth. in Bull. Torrey Bot. Club **33**: 31. 1906, based on uredia.

Teliis hypophyllis, aggregatis, 0.35–0.5 mm diam., pulvinatis, ceraceis, ochraceo-brunneis; teliosporis cylindraceis vel plus-minusve clavatis, 11–18 × 54–74 μ, membrana hyalina, ca. 1 μ cr., ad apicem 6–14 μ.

TYPE: On *Eupatorium lindleyanum* DC., Ch'ing Yang Hsien, Anhwei Prov., China, Oct. 14, 1932, S. Y. Cheo *1240*.

Telia were first described by Tai (Farlowia **3**: 101. 1947) but without Latin, hence the name was not validated by his publication.

Cronartium fusiforme Hedgc. & Hunt sp. nov.

Peridermium fusiforme Arth. & Kern in Bull. Torrey Bot. Club **33**: 421. 1906; *Cronartium fusiforme* Hedgc. & Hunt in Phytopathology **8**: 316. 1918 *nom. nud.*

Urediis hypophyllis, sparsis, minutis, poro centrali apertis, peridio tenui; urediosporis plerumque ovatis vel obovatis, (11–)13–16(–18) × (14–)17–24(–28) μ; membrana hyalina, echinulata, 2–2.5(–3) μ cr., poris germ. obscuris. Teliis hypophyllis, cylindraceis vel filiformibus, brunneis, usque ad 3 mm longis; teliosporis oblongis vel oblongo-fusiformibus, variabiles, (10–)12–16(–20) × (25–)30–44(–54) μ, aureis, levibus.

TYPE: On *Quercus nigra* L., Brooksville, Florida, Mar. 23, 1914, W. H. Long (Forest Path. No. *15170*). (Type in the National Fungus Collections, Beltsville, Md.)

Gymnosporangium hyalinum Kern sp. nov.

Roestelia hyalina Cke. in Bull. Soc. Bot. Fr. **24**: 315. 1877; *Aecidium hyalinum* (Cke.) Farl. in Bibl. Index **1**: 55. 1905; *Gymnosporangium hyalinum* Kern in Bull. N. Y. Bot. Gard. **7**: 470. 1911, based on aecia.

Teliis caulicolis, in incrassationibus fusiformibus vel nullis aggregatis, pulvinatis, brunneis, 1–5 mm diam. vel confluentibus; teliosporis ellipsoideis, 1-septatis, 16–21 × 40–63 μ, membrana aureo-brunnea, 1–1.5 μ cr., ad apicem usque ad 3–4 μ cr., poris germ. apicalis; pedicello longo, hyalino, 3–5 μ diam.

TYPE: On *Chamaecyparis thyoides* (L.) B.S.P., 3½ miles east of Bristol, Fla., March 4, 1946, F. D. Kern, C. R. Orton and E. West. (Type in Pennsylvania State University Herbarium, University Park.)

Telia were first described by Kern and West (Mycologia **39**: 123. 1947) but without Latin.

Phakopsora antiguensis Kern et Thurston sp. nov.

Uredo antiguensis Cumm. in Bull. Torrey Bot. Club **67**: 613. 1940; *Phakopsora antiguensis* (Cumm.) Kern & Thurston in Mycologia **36**: 508. 1944 *nom. nud.*

Teliis hypophyllis, subepidermalibus, indehiscentibus, uredosoros circumdantibus, atro-brunneis, 0.1–0.5 mm diam., ex sporis 2–3 irregulariter superpositis compositis; teliosporis cuboideis vel oblongis, 10–13 × 16–26 μ, membrana cinnamomeo-brunneis, 1.5 μ cr. vel ad apicem lenissime incrassata.

TYPE: On *Acalypha* sp., Road Petare-Guarenas, Est. Miranda, Guatemala, Mar. 15, 1939, Whetzel & Muller *2972*. (Type in Pennsylvania State University Herbarium, University Park.)

The uredial stage was described from Guatemalan material. Kern and Thurston did not provide a Latin description.

Phakopsora cherimoliae Cumm. sp. nov.

Uredo cherimoliae Lagh. in Bull. Soc. Mycol. Fr. **11**: 215. 1895; *Uredo cupulata* Ell. & Ev. in Field Mus. Publ. Bot. **2**: 16. 1900; *Physopella cherimoliae* (Lagh.) Arth. in Rés. Sci. Congr. Bot. Vienne p. 338. 1906, based on uredia; *Phakopsora cherimoliae* Cumm., in Bull. Torrey Bot. Club **68**: 467. 1941 *nom. nud.*

Teliis hypophyllis, subepidermalibus, indehiscentibus, atro-brunneis, ex sporis 3–6 irregulariter superpositis compositis; teliosporis cuboideis, oblongis vel oblongo-ellipsoideis, 7–13 × 13–23 μ, membrana 1–2 μ cr. vel ad apicem lenissime incrassata, aureo- vel castaneo-brunneis.

TYPE: On *Anona cherimolia*, Moran, Guatemala, Feb. 11, 1905.

Cummins' original description of the telia was in English.

Phakopsora jatrophicola Cumm. sp. nov.

Uredo jatrophicola Arth. in Mycologia **7**: 331. 1915; *Phakopsora jatrophicola* Cumm. in Bull. Torrey Bot. Club **64**: 43. 1937 *nom. nud.*

Teliis hypophyllis, subepidermalibus, indehiscentibus, atro-brunneis, ex sporis 5–12 irregulariter superpositis compositis; teliosporis cuboideis vel oblongis, 7–13 × 13–27 μ, membrana castaneo-brunneis, 1 μ cr. vel apicem lenissime incrassatis.

TYPE: On *Jatropha canescens* Muell.-Arg., in the Laguna Mountains east of Todos Santos, Lower California, Mexico, Feb. 21, 1928, Marcus Jones *24531*.

Cummins' original description of the telia was not in Latin.

Puccinia ammophilina Mains sp. nov.

Uredo ammophilina Kleb. in Kryptog. Fl. Mark Brandenburg **5A**: 882. 1914; *Puccinia ammophilina* Mains in Bull. Torrey Bot. Club **66**: 617. 1939 *nom. nud.*

Teliis epiphyllis, castaneo-brunneis, tarde nudis; teliosporis clavatis vel oblongo-clavatis, 16–22 × 38–62 μ, membrana 1 μ cr., flavo-brunnea, ad apicem 4–5 μ cr. et castaneo-brunnea; pedicello brevi.

TYPE: On *Ammophila arenaria* (L.) Link, Lake Lytle, Oregon, Nov. 4, 1936, R. Sprague (Ore. No. *10–733*). (Type in the University of Michigan Herbarium, Ann Arbor.)

Mains' original description was not in Latin.

Puccinia costina Cumm. sp. nov.

Uredo costina Syd. in Ann. Mycol. **14**: 355. 1916; *Puccinia costina* (Syd.) Cumm. in Mycologia **33**: 381. 1941 *nom. nud.*

Teliis minutis, 50–80 μ diam., dense aggregatis, erumpentibus; teliosporis ellipsoideis vel oblongo-ellipsoideis, ad septum non vel vix constrictis, 9–14(–16) × 23–30 μ, membrana hyalina, uniformiter 0.5 μ cr., levi; pedicello hyalino, brevi; statim germ.

TYPE: On *Costus* sp., Boana, Morobe, New Guinea, May 10, 1940, Clemens *11368*.

Cummins' first description of telia was not in Latin.

Puccinia degener Mains & Holw. sp. nov.

Puccinia (?) *degener* Mains & Holw. ex Arthur in Am. Jour. Bot. **5**: 482. 1918, based on uredia; *Uredo degener* (Mains & Holw.) Arth. in N. Am. Flora **7**: 616. 1924.

Teliis hypophyllis, sparsis, rotundatis, 0.1–0.3 mm diam., pulvinatis, cinnamomeis, teliosporis ellipsoideis vel oblongo-ellipsoideis, (20–)23–26 × 36–43(–46) μ, membrana 1.5 μ cr., cinnamomeis vel aurea-brunneis, poris germ. apicalis, in cellulis apicalis papilla hyalina 3 μ cr provisis, pedicello hyalino, fragile; statim germ.

TYPE: On *Salvia myriantha* Epling, region of Las Nubes, so. of San Martin Chile Verde, Guatemala, Jan. 16, 1941, Standley *83614*.

Cummins (Bull. Torrey Bot. Club **70**: 75. 1943) first reported telia (and aecia) but without a Latin description.

PUCCINIA JALAPENSIS (Holw.) Barth. in N. Am. Ured. No. 2549. 1922.

Aecidium jalapense Holw. in Ann. Mycol. **2**: 392. 1904, with undescribed telia; *Dicaeoma jalapense* (Holw.) Arth. in N. Am. Flora **7**: 402. 1920.

Teliis non visis. Teliosporis ellipsoideis vel clavato-ellipsoideis, ad septum leniter constrictis, 17–23 × 46–58 μ, membrana 1–1.5 μ cr., ad apicem 4–7 μ, aureo-brunnea, levi, poris germ in cellulis superioribus apicalibus, inferioribus juxta septum dispositis; pedicello hyalino, persistenti, usque ad 55 μ longo.

Both N. Am. Ured. Nos. *1501* and *2549* are from Holway's original collection No. *3076* on *Ipomoea jalapa* (L.) Pursh, Jalapa, Mexico,

Oct. 5, 1898. A few teliospores are present, hence Holway's *Aecidium jalapense* is based on a type bearing the perfect state although not described. A description of the teliospores is given above.

Puccinia neorotundata Cumm. nom. nov.

Puccinia rugosa Speg. in An. Soc. Cient. Argent. **17**: 92. 1884, non Billings 1871; *P. rotundata* Diet. in Hedwigia **36**: 32. 1897, non Bonorden 1860; *Micropuccinia rotundata* (Diet.) Arth. & Jacks. in N. Am. Flora **7**: 572. 1922.

Puccinia biocellata Vest. sp. nov.

Uredo plucheae Syd. in Ann. Mycol. **1**: 333. 1903; *Uredo biocellata* Arth. in Bull. Torrey Bot. Club **33**: 517. 1906; *Puccinia biocellata* (Arth.) Vest. in Micromycetes rar. sel. Nos. *1267, 1368*. 1908 *nom. nud.; Uredo plucheae* Speg. in An. Mus. Nac. Buenos Aires **19**: 319. 1909; *Puccinia plucheae* (Syd.) Arth. in Bull. Torrey Bot. Club **49**: 194. 1922 *nom. nud.; Dicaeoma plucheae* (Syd.) Arth. & Jacks. in N. Am. Flora **7**: 793. 1926.

Teliis hypophyllis, sparsis, rotundatis, 0.2–0.5 mm diam., pulvinatis, cinnamomeo-brunneis; teliosporis ellipsoideis vel oblongo-ellipsoideis, utrinque plerumque contractis, medio non vel vix constrictis, (16–)18–21(–23) × (40–)43–55(–60) μ; membrana aureo-brunnea, levi, 1–1.5 μ cr., ad apicem umbonata 5–8 μ cr.; poris germ. in cellulis superioribus apicalibus, inferioribus juxta septum dispositis; pedicello hyalino, usque ad 65 μ longo, semipersistenti; statim germ.

TYPE: On *Pluchea fastigiata* Griseb., Quinta near Laguna de la Brea, Prov. Jujuy, Argentina, June 13, 1901, Vestergren (Micromycetes rar. sel. No. *1368*).

Vestergren published no description of the teliospores although a few are present in his specimens. Hence the name cannot be displaced by *P. plucheae* Arth., which was based on the same material and also published without a description of the telial stage.

Roestelia guatemaliana (Crowell) Cumm. comb. nov.

Gymnosporangium guatemalianum Crowell in Can. Jour. Res. C. **18**: 11. 1940, based on aecia.

Scopella bauhiniicola Cumm. sp. nov.

Uredo bauhiniicola P. Henn. in Hedwigia **34**: 98. 1895; *Scopella bauhiniicola* (P. Henn.) Cumm. in Bull. Torrey Bot. Club **67**: 72. 1940 *nom. nud.*

Teliis hypophyllis, ceraceis, flavidis, pulvinatis, 0.1–0.3 mm diam.; teliosporis 5–7 ex cellulis basalibus natis, ellipsoideis vel oblongo-ellipsoideis, 13–18 × 30–45 μ,

membrana uniformiter 1–1.5 μ cr., poris germ. nullis; pedicello sporam aequante vel longiore, 13–20 μ lato, hyalino; statim germ.

TYPE: On *Bauhinia heterophylla* Kunth, Canasi, Prov. Matanzas, Cuba, Mar. 25, 1919, J. R. Johnston *1115*.

Cummins' original description of the telia was not in Latin.

Scopella sapotae Mains sp. nov.

Uredo sapotae Arth. & J. R. Johnston in Mem. Torrey Bot. Club **17**: 169. 1918; *Scopella sapotae* (Arth. & J. R. Johnston) Mains in Ann. Mycol. **37**: 59. 1939 *nom. nud.*

Teliis hypophyllis ceraceis, 0.1–0.2 mm diam.; teliosporis obovoideis, 16–22 × 32–40 μ, membrana uniformiter 1 μ cr., pallide flavidis, poris germ. nullis; pedicello hyalino, persistenti, usque ad 20 μ longo; statim germ.

TYPE: On *Achras zapota* L., Valentin, El Cayo Distr., British Honduras, June 25, 1936, E. B. Mains *3600*. (Type in the University of Michigan Herbarium, Ann Arbor.)

Mains' description of telia was not in Latin.

Uraecium extensum (Arth.) Cumm. comb. nov.

Pileolaria extensa Arth. in N. Am. Flora **7**: 148. 1907, based on uredinoid aecia.

Uredo acaciae-burseriae Cumm. nom. nov.

Ravenelia inquirenda Arth. & Holw. in Arthur in Am. Jour. Bot. **5**: 423. 1918, based on uredia; not *Uredo inquirenda* Arth. 1907.

Uredo adenocaulonis (Jacks.) Cumm. comb. nov.

Coleosporium adenocaulonis Jacks. in Brooklyn Bot. Gard. Mem. **1**: 202. 1918, based on uredia.

Uredo alaskana (Mains) Cumm. comb. nov.

Pucciniastrum alaskanum Mains in Bull. Torrey Bot. Club **66**: 621. 1939, based on uredia.

Uredo arida (Jacks. ex Arth.) Cumm. comb. nov.

Coleosporium aridum Jacks. ex Arthur in Bull. Torrey Bot. Club **51**: 52. 1924, based on uredia.

Uredo consimilis (Arth.) Cumm. comb. nov.

Milesia consimilis Arth. in Mycologia **7**: 176. 1915, based on uredia.

Uredo cubensis (Arth. & J. R. Johnston) Cumm. comb. nov.

Ravenelia cubensis Arth. & J. R. Johnston in Mem. Torrey Bot. Club **17**: 118. 1918, based on uredia.

Uredo hoffmanseggiae (Long) Cumm. comb. nov.

Ravenelia hoffmanseggiae Long in Bot. Gaz. **64**: 57. 1917, based on uredia.

Uredo investita (Faull) Cumm. comb. nov.

Uredinopsis investita Faull in Contr. Arnold Arbr. No. 11: 35, 1938, based on uredia.

Uredo mexicana (Arth.) Cumm. comb. nov.

Pileolaria mexicana Arth. in N. Am. Flora **7**: 149. 1907, based on uredia.

Uredo neopustulata Cumm. nom. nov.

Uredo pustulata P. Henn. in Hedwigia Beibl. **35**: 129. 1899; not *Uredo pustulata* Pers. 1801.

Uredo obovata (Arth.) Cumm. comb. nov.

Desmella obovata Arth. in Mycologia **21**: 78. 1929; *Hyalopsora obovata* (Arth.) Cumm. in Ann. Mycol. **38**: 336. 1940, based on uredia.

Uredo piscariae (Jacks.) Cumm. comb. nov.

Melampsora piscariae Jacks. in Brooklyn Bot. Gard. Mem. **1**: 212. 1918, based on uredia.

Uredo spigeliae (Arth.) Cumm. comb. nov.

Coleosporium spigeliae Arth. in Bull. Torrey Bot. Club **51**: 51. 1924, based on uredia.

Uromyces eugentianae Cumm. nom. nov.

Uromyces gentianae Arth. in Bot. Gaz. **16**: 227. 1891 non Léveille 1847.

Inasmuch as *Uredo gentianae* Strauss (= *Puccinia gentianae* (Strauss) Roehl.) was based on a specimen including telia, Léveille's (Ann. Sci. Nat. Bot. Ser. 3. **8**: 371. 1847) combination as *Uromyces gentianae* (Strauss) Lév. must be considered as a valid transfer.

THE ARTHUR HERBARIUM,
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LAFAYETTE, INDIANA

A ROTARY SHAKING MACHINE FOR LABORATORY USE[1]

LEO KAPLAN

(WITH 3 FIGURES)

In the study of mold metabolism, it is frequently desirable to grow organisms in submerged culture in order to have physiologically homogeneous mold material. Following the current "do it yourself" philosophy, plans are here presented for the construction of a relatively cheap and simple rotary shaking machine. The rotary or swirling action has the advantage over reciprocal action of keeping fungi submerged and preventing their growth and sporulation on the sides of the flask. The rotary motion also ensures adequate aeration of cultures and good contact between metabolizing hyphae and the medium.

As shown in FIG. 1, the machine consists of a lower stationary platform, a central moveable platform and an upper moveable tray. The central platform is able to move in a north-south direction on the lower stationary platform while the upper tray is able to move in an east-west direction on the central platform. With this arrangement, the top tray is free to move in any direction by combining its action with that of the central platform. Thus, when a motor-driven eccentric is attached to the tray, the movement described by the tray will be that of the eccentric.

The bearing surfaces of the platform and tray consist of V-shaped runners. One runner is placed at each of the four corners of the lower platform and each is oriented north-south. An opposing set is attached to the bottom of the central platform in line with the set on the lower platform. Another set of runners, oriented east-west, is attached to the upper side of the central platform. This set is opposed by a set of runners attached to the bottom of the tray. A small steel ball-bearing is placed between each opposing pair of runners. FIG. 2 shows an opposing pair of runners. Note that the lower runner of each pair is made with a pin at each end to prevent the ball bearing from sliding out the end of the runner while the machine is in motion.

The runners can be made of one-half inch cold rolled steel and can be case hardened and ground after machining. Each runner has a

[1] This work was supported by a grant from the Graduate Council of Southern Illinois University.

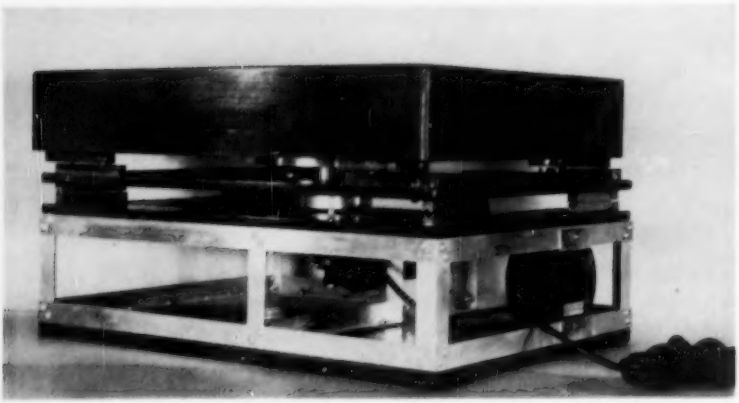
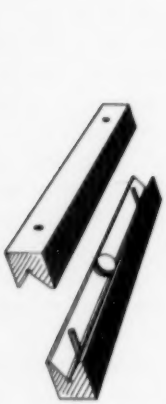


FIG. 1. Assembled shaking machine.

V-shaped groove running the entire length of the piece. These runners may be used without hardening but will eventually show wear under these conditions.

The lower platform is bolted to a frame made of aluminum angle which can be purchased at most hardware or building supply outlets. Mounted on this aluminum frame is a stirring motor with a geared drive and a rheostat for speed control. Motors of this type can be obtained from almost any scientific apparatus company.



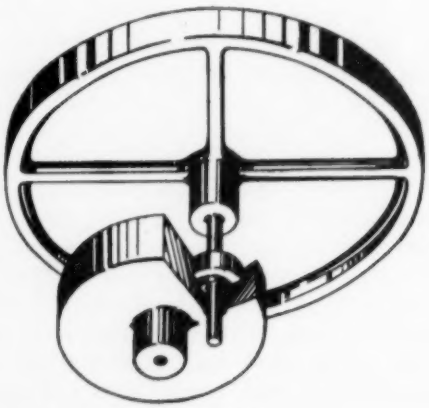


FIG. 2. Detail of a set of runners (left).
FIG. 3. Detail of driving eccentric (right).

The driving assembly, illustrated in FIG. 3, consists of a metal disc mounted on the motor shaft. This disc has a bearing mounted in an eccentric position. The distance of this bearing from the center will determine the radius described by the tray. It has been found that a short radius gives ample swirling action and reduces the centrifugal force, thus reducing the tendency of the machine to creep. Large suction cups may also be attached to the bottom of the machine to further anchor the entire assembly.

Another metal disc having a short shaft is mounted on the underside of the tray. This shaft projects through the eccentric bearing when the machine is assembled. The center portions of the lower and central platforms must be cut away to allow the driving assembly to engage the shaft attached to the bottom of the tray.

The shaker can be made in any desirable size. The dimensions of the shaker illustrated in FIG. 1 are 18″ × 24″ for the tray and an over-all height of 10″. This tray will accommodate forty-eight 125 ml flasks. If more shaking area is desired, a second tray can be attached above the first.

Other means of driving the shaker can be devised. A split sheave arrangement can be used and, although it makes the entire assembly more bulky, it allows the use of a sturdier and cheaper motor. If variable speed is not important, it is possible to use a gear reduction unit of specific speed.

A shaker using a gear reduction unit and driven by a ¼ H.P. motor has been in use in this laboratory for the past three years and has required a minimum of attention even on extended runs.

In general, the rotary shaking machine described in the foregoing operates well for extended periods of time and requires little or no servicing.

DEPARTMENT OF BOTANY,
SOUTHERN ILLINOIS UNIVERSITY,
CARBONDALE, ILLINOIS

NOTES AND BRIEF ARTICLES

A SLIME MOLD IN AN UNUSUAL HABITAT

Stemonitis webberi Rex was found on the bottom edge and sides of wooden beams of the ceiling of a rather humid room at Chas. Pfizer & Co., Inc. The presence of the slime mold here has been known since 1947 or 1948. It has been seen rarely in the summer, but it is frequently observed during the rest of the year. Presumably the condensation of steam on the cooler ceiling has been conducive to growth of the organism. At present, it occurs in spots perhaps a foot apart over approximately 500 sq ft of ceiling.

At various times phenol, formaldehyde and a 50% caustic solution have been used to wash off the slime mold before the beams are repainted. The organism always has reappeared.

The diagnostic features of the specimens collected here have agreed nicely with those given for this species by Macbride and Martin in their book, *The Myxomycetes*. There was one noteworthy difference. The tip of each sporangium was enlarged to nearly 1 mm and was firm, though easily crushed. This tip consisted of amorphous material. The tips of adjoining sporangia were fused to form a crust. Probably this development was due to the fact that the specimens developed in a pendant position from the bottom of a beam. Unfortunately, no specimens from the sides of the beams were available for examination. The plasmodia have not been observed.

The existence of this slime mold in the plant was brought to my attention by Mr. Dan Badia.—JOHN B. ROUTIEN, Biochemical Research Department, Chas. Pfizer & Co., Inc., 630 Flushing Avenue, Brooklyn 6, New York.

A NOTE ON SPORONEMA PULVINATUM SHEAR

In the paper, studies in the genus *Sporonema*,[1] it was stated that only *Sporonema oxycocci* Shear was found on the type material of *Sporonema pulvinatum* Shear. The opinion was expressed that the original fungus studied by Shear had been used up or lost. Due to

[1] Limber, Donald P. Studies in the genus *Sporonema*. Mycologia **47**: 389–402. 1955.

the excellent memory of Dr. Shear and the kindness of Miss E. K. Cash the original slides on which *S. pulvinatum* was based have been found in the National Fungus Collections at Beltsville, Md. The material on these slides is typical of *Sclerotiopsis concava* Desm., the species to which *S. pulvinatum* was transferred by Shear and Dodge.[2]—DONALD P. LIMBER, U. S. Department of Agriculture, Agricultural Research Service, Plant Quarantine Branch, Hoboken, N. J.

A NEW SPECIES OF MICROSPORUM

In the Journal of Investigative Dermatology **23**: 51–61. 1954, Fuentes, Aboulafia and Vidal described what was regarded as a variety of *Microsporum gypseum*. This organism was designated as var. *nana*, but no formal description nor Latin diagnosis was given. It had the following history and characteristics:

1. This fungus had been isolated from a kerion that had developed on the scalp of a boy.

2. It invaded hair as an endothrix organism.

3. It had been isolated in pure culture and experimentally demonstrated to be pathogenic to guinea pigs and a human.

4. The organism was recovered from the experimentally induced lesions.

The colonies grow rapidly, are floccose white at first but orange on the reverse side, finally granular but buff colored and brownish red on the reverse side. At maturity tufts appear at the surface. The macroconidia are thick walled, finely verrucose, sometimes smooth, numerous, ovoid or rarely spherical, or else elliptical, 12–18 × 7.5 μ, non-septate to 1–2-septate, rarely 24 μ long and 3–4-celled. The microconidia are sessile, clavate to cylindrical, 5 × 2 μ. Racquet hyphae, intercalary chlamydospores, pectinate hyphae, and loosely coiled hyphae are rare.

Seven months after reporting on this "dwarf" form of *M. gypseum* a similar strain was recovered from a glabrous skin infection in an adult. This second isolate behaved as did the first one.

These two isolates have proved to be stable in their gross and microscopic characteristics over a period of three years. In view of this and the fact that its macroconidia are fundamentally different from those

[2] Shear, C. L. and B. O. Dodge. The life history and identity of *"Patellina fragariae," "Leptothyrium macrothecium,"* and *"Peziza oenotherae."* Mycologia **13**: 135–170. 1921.

produced by *M. gypseum* it is proposed that this organism now be considered a new species:

Microsporum nanum sp. nov.

Coloniae rapide crescant ac floccosae sunt, ab initio albae, sed ad laterum adversum aureae, deinde granulosae, luteae, atque ad laterum adversum brunneorubricosae. Ad maturitatem coloniae cristatae sunt. Macroconidiis pachydermaticis, subtiliter verrucosis interdum levibus, numerosis, ovoideis vel raro sphaericis

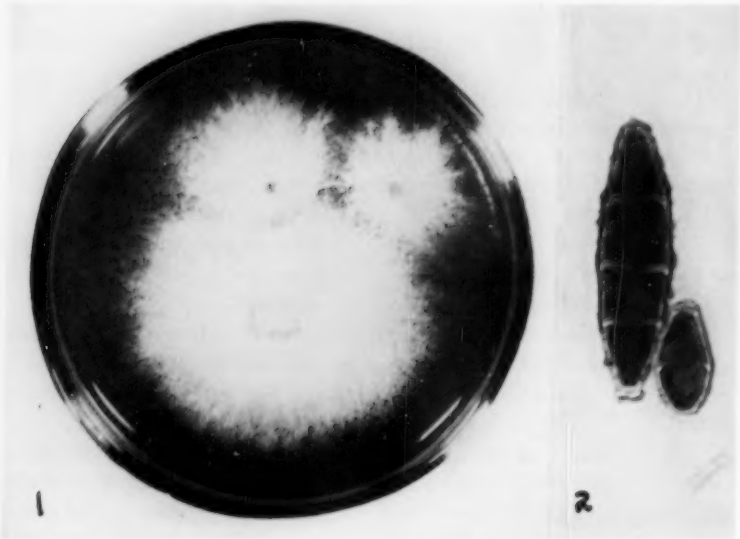


FIG. 1. *Microsporum nanum*. Colony on Sabouraud agar.

FIG. 2. Macroconidia of *Microsporum gypseum* and *M. nanum* showing difference in size.

vel ellipticis, 12–18 × 5–7.5 μ, non septatis vel 1–2-septatis, raro 24 μ longis atque 3–4-cellulosis. Microconidiis sessilibus, clavatis vel cylindricis, 5 × 2 μ. Hyphis "racquet," chlamydosporis intercalaribus, hyphis pectinatis, ac hyphis in spiram convolutis, raris.

This fungus can be obtained from the Centralbureau voor Schimmelcultures, American Type Culture Collection, Communicable Disease Center, U.S.P.H.S., Atlanta, Ga., and from our collection.

I am indebted to Dr. Frederick A. Wolf of Duke University for the Latin transcription.—CÉSAR A. FUENTES, Escuela de Medicina, Havana, Cuba.

REVIEWS

THE FUNGUS FLORA OF THE LENINGRAD REGION, I, by N. A. Naumov. U.S.S.R. Academy of Sciences, Moscow-Leningrad, 182 pp., 102 figures (Russian), 1954. (Price 10 roubles 60 kopeks.)

Since the translation into French of N. A. Naumov's treatment of the Mucorales (Clés des Mucorinées, Paul Lechevalier, Paris, 1939), nothing like a comprehensive study of this order has appeared in Russian until this book appeared in 1954. This book is not quite as comprehensive in scope as the title implies for it deals exclusively with Phycomycetes but presumably is the first in a series of volumes on fungi. There are 182 pages of descriptions (73 genera and 312 species) and 102 line drawings which are in general quite satisfactory. Keys to the recognized orders, families and genera are given. Naumov's scheme of classification is similar to Gäumann's. The book begins with a short introduction and then treats the Class Archimyceteae which contains the orders Plasmodiophorales, Chytridiales, Mycochytridiales, Woroninales and Ancylistales. The last two orders belong in the group Biciliatae, the remainder in the Uniciliatae. The second class, Phycomyceteae, is divided into two subclasses, Oomyceteae and Zygomyceteae. The subclass Oomyceteae is divided into the Uniceliatae containing the order Monoblepharidales and the second, Biciliatae, containing the orders Saprolegniales, Blastocladiales and a third, Aciliatae, with the order Peronosporales. The subclass Zygomyceteae contains the orders Mucorales and Entomophthorales. The system of arrangement of the Mucorales is similar to that in the 1939 book listed above except that the genus *Naumoviella* Novotelnova (Family Mortierellaceae) with its four species, three of which are new, is added. Several other new species, varieties and at least one subgenus are erected and lists of hosts are given. The book is written in Russian except for the Latin names.—C. W. HESSELTINE.

BIBLIOGRAPHIE DER PFLANZENSCHUTZ-LITERATUR, 1951, by J. Bärner. xliv + 420 pp. Biologische Bundesanstalt für Land- und Forstwirtschaft in Berlin-Dahlem. Verlag Paul Parey, Berlin. 1955.

Thorough and well-ordered bibliographies are welcome tools for the investigator, and in this continuation of the coverage of plant patho-

logical literature we have such an implement. The arrangement of the citations is similar to that of the earlier volumes, consisting of a "General Part" covering text-books, governmental and societies' reports, bibliographies and biographies; a section on "Diseases and causes" arranged by cause; a section on "Disease and host plants" arranged by groups of host-plants and a section on "Measures of plant protection." An author index concludes the volume. With this volume the phytopathological literature from 1914 to 1951 has been compiled, with the exception of the years 1946–50. The author promises coverage of this gap in the near future. On the whole the work is carefully done and the coverage remarkably complete, although minor errors of translation do occur; for example a paper on diseases of "cane-fruits" is cited in the section on sugar cane diseases.—JOSEPH C. GILMAN.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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